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(54) Title: PHARMACEUTICAL COMPOSITION CONTAINING USCHARIDIN OR ITS ANALOGUES

(57) Abstract

The invention provides compositions comprising uscharin and the use of uscharin to combat cell proliferation for example in the treatment of cancer. Administration of uscharin may kill or reduce the growth rate of cancer cells and may also be of application in other medical conditions presenting symptoms of excessive or uncontrolled cell proliferation. The composition may be administered by any convenient route and formulated accordingly. The composition may be administered locally or generally and may be suitably dissolved and/or suspended in a pharmaceutically acceptable liquid carrier medium.

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PHARMACEUTICAL COMPOSITION CONTAINING USCHARIDIN OR ITS ANALOGUES This invention relates to a composition comprising the cardenolide glycoside uscharin. Plants of the family Asclepidaceae are known to be extremely poisonous. Such plants have a history of use in folk medicines in those areas where they occur 8 naturally, for example in South East Asia and Africa. Two of the best known representatives of the 10 Asclepiadaceae are Calotropis gigantea and Calotropis 11 procera. Extracts from Calotropis procera plants have 12 traditionally been used as an abortifacient, for 13 14 infanticide, for rheumatic pain and to produce a 15 purgative. 16 The stems, flowers and leaves of plants from the family 17 18 Asclepiadaceae (including Calotropis gigantea and 19 Calotropis procera) are known to contain certain 20 compounds known as cardenolides. In several species 21 substantial amounts of cardenolides have been found to 22 be concentrated in the latex (Roeske et al, in 23 Biochemical Interactions Between Plants and Insects 24 published in Volume 10 of Recent Advances in

Phytochemistry, Plenum Press, New York (ed. Wallace), 1 Seiber et al, Phytochemistry 2:1:2343 (1982), Seiber et 2 al, in Isopentoids in Plants, Academic Press (ed Nes, 3 1984) and Seiber et al, in J. Chem. Ecol.  $\underline{6}$ :321 (1980)). The natural production of cardenolides in 5 Ascelopias curassavia has been reported by Groeneveld et al in Phytochemistry 29(11):3479-3486 (1990). 7 Examples of cardenolide glycosides found in C. procera 8 are voruscharin, uscharin, uscharidin, calotropin, 9 calactin, calotoxin, and calotropagenin. Formula I 10 shows the chemical structure of these cardenolides. 11

It has now been found that the cardenolide uscharin is 1 2 particularly useful for medical purposes. Whilst 3 uscharin has been isolated and its chemical structure determined, no utility for this compound has previously 4 been reported. 5 6 The present invention thus provides a composition 8 comprising uscharin, the analogues and salts thereof as 9 active ingredient together with a pharmaceutically acceptable carrier or excipient. 10 11 12 Further, the present invention also provides the use of 13 uscharin, the analogues and salts thereof for medical 14 (including veterinary) purposes. 15 16 Previously, certain cardenolide glycosides such as calotropin and uzarigenin have been noted to have 17 18 cytotoxic activity against primate tumour cells. 19 Certain cardenolide glycosides from the Asclepiadaceae 20 family share structural and pharmacological 21 similarities with the Digitalis cardiac glycosides. 22 Whilst we do not wish to be bound by theoretical 23 considerations it is believed that the cytotoxicity of 24 some cardenolide glycosides is related to the 25 inhibition of the plasma membrane bound Na<sup>+</sup>/K<sup>+</sup> ATPase 26 (ie analogous to the manner in which Digitalis cardiac 27 glycosides exert their toxic effects). However, it has 28 also been shown that whilst some cardenolide glycosides 29 are cytotoxic to cell cultures they have no in vivo 30 tumour-inhibiting activity. This is true of calotropin 31 and uzarigenin. 32 33 It has never previously been proposed that uscharin 34 would be useful for medical applications. The 35 inventors' results have shown that at lmg/ml a primary

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extract of Calotropis gigantea known as CGE-1 does have 1 tumour inhibiting activity in rats (weighing about 2 200g) and does not lead to the death of the test 3 animals. 4 5 Typically, the use of uscharin according to the present 6 invention is to combat cell proliferation for example 7 in the treatment of cancer. Thus administration of 8 uscharin may kill or reduce the growth rate of cancer 9 cells and may also be of application in other medical 10 conditions presenting symptoms of excessive or 11 uncontrolled cell proliferation. 12 13 The word "combat" is used herein to refer to treatment 14 of an existing condition so as to alleviate or reverse 15 the symptoms of the condition in an affected human or 16 animal and to prevent such a condition in a healthy 17 human or animal. 18 19 The composition according to the present invention may 20 be administered by any convenient route and mention may 21 be made of enteral, parenteral, topical administration 22 and the composition will be formulated accordingly. 23 Conveniently, the composition may be administered 24 locally to the affected site, generally by means of 25 injection. Thus the uscharin will be suitably 26 dissolved and/or suspended in a pharmaceutically 27 acceptable liquid carrier medium, which will generally 28 be aqueous-based, for example an isotonic solution. 29 Alternatively, the composition according to the 30 invention may be taken orally. 31 32 Formulations for parenteral administration include 33 aqueous and non-aqueous isotonic sterile injection 34

solutions which may contain anti-oxidants, buffers,

bacteriostats and solutes which render the formulation 1 isotonic with the blood of the intended recipient; and 2 aqueous and non-aqueous sterile suspensions which may include suspending agents and thickening agents. formulations may be presented in unit-dose or multi-5 dose sealed containers, for example, ampoules and 6 vials, and may be stored in a freeze-dried 8 (lyophilized) condition requiring only the addition of .... the sterile liquid carrier, for example water for 10 injections, immediately prior to use. Extemoraneous 11 injection solutions and suspensions may be prepared 12 from sterile powders, granules and tablets of the kind 13 previously described. 14 The dose will depend on a number of factors known to 15 16 the skilled physician including the severity of the conditions, the identity of the recipient; and also the efficacy and toxicity of the particular composition which is being administered. Generally doses in the range 0.1-100 mg/kg body weight may be used,

17 18 19 20 21 particularly 1-10 mg/kg. The frequency of 22 administration will vary depending on the rate of .23 metabolism or excretion of the administered compound, 24 but may be repeated daily, optionally as two or more 25 sub-doses. Unit doses of 20 to 500 mg, preferably 100 26 to 400 mg may be used.

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A single dosage may be given daily or smaller quantities or dosage units may be given at intervals throughout a 24 hour period, for example dosage units given 2, 3 or 4 times throughout the day.

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Any type of cancer or condition involving cell proliferation may be treated by the present invention. Uscharin is especially useful for the treatment of

cancers such as leukaemia, non-small cell lung cancer, 1 small cell lung cancer, colon cancer, CNS cancer, 2 melanoma, ovarian cancer, renal cancer, prostrate 3 cancer, and breast cancer. However the invention is 4 ' not limited to treatment of these specific conditions 5 since uscharin is believed to be of general effect. 6 7 Cancers where uscharin is particularly efficacious 8 include ovarian cancer and skin cancer. 9 10 Uscharin may by produced by any convenient method, for 11 example by chemical synthesis. Alternatively the 12 uscharin may be conveniently extracted and purified 13 from organisms (for example plants of the family 14 Asclepiadacaeae) which produce uscharin naturally. It 15 is also envisaged that uscharin may be manufactured 16 using genetically engineered micro-organisms, plants or 17 animals or may be made using cell-culture or other 18 biotechnological techniques. 19 20 Further, the present invention also provides the use of 21 a composition as described above for medical purposes, 22 for example to combat conditions in which cell 23 proliferation is undesirable (eg cancer). 24 25 In another aspect, the present invention provides the 26 use of uscharin in the manufacture of a medicament. 27 Generally such medicament would be of use to combat 28 cancer and other conditions where cell proliferation is 29 undesirable. 30 31 In a further aspect, the present invention provides a 32 method of treatment of a human or non-human animal 33 body, said method comprising administering to said body 34 a composition as described above. 35

The present invention is now further described by means of the following, non-limiting Examples. EXAMPLE 1 5 PREPARATION OF USCHARIN EXTRACT 6 7 ISOLATION OF CGE-1 8 (i) 9 Leaves of Calotropis gigantea (500g) were Soxhlet 10 extracted initially with petroleum ether (60-80), then 11 ethyl acetate and finally methanol. The cell culture 12 13 bioassays showed that the ethyl acetate fraction contained cytotoxic activity. The ethyl acetate 14 extract was subjected to vacuum liquid chromatography 15 (VLC) on silica gel 60H (Merck). Elution was initiated 16 17 with petroleum ether (60-80) and proceeded with 18 petroleum ether containing progressively greater 19 amounts of ethyl acetate through to ethyl acetate only. 20 Elution was then continued with ethyl acetate containing progressively greater amounts of methanol. 21 22 23 Samples of the fraction were collected and prepared for 24 cytotoxicity testing by solubilisation in 0.1% Tween. 25 26 The greatest cytotoxic activity (ED<sub>50</sub> <  $0.10\mu g/ml$ ) was 27 found in the 70-80% ethyl acetate in petroleum ether 28 fractions. The cytotoxic compound CGE-1 (72.0 mg) 29  $(ED_{50} < 0.09 \mu g/ml)$  was isolated as a white semi-30 crystalline precipitate from this fraction. 31 32 (ii) ISOLATION OF CGE-2 33 34

Another less cytotoxic compound, CGE-2 (101.0mg) (ED<sub>50</sub>

<8.0µg/ml) was isolated from the 100% ethyl acetate

```
fraction as a semi-crystalline precipitate.
 1
                PROPERTIES OF CGE-1
      (iii)
 4
      White powder, found 587.2511, C31H41NO8S requires
 5
      587,2553. [\propto]_0 + 10.0^{\circ} (c.0.1, CH_3OH_4) IR
 6
      V_{max} CM<sup>-1</sup>: 3465, 2960, 2920, 2840, 2720, 1735, 1730,
 7
      1705, 1625, 1540, 1160, 1110, 1060, 1040. EIMS m/z
8
      (rel. int.) 587 [M+] (4.0), 233 (14.9), 215 (8.6), 187
      (9.8), 183
10
11
12
      ACTIVITY OF CGE-1
13
      At a concentration of 1 mg/ml, CGE-1 has a tumor
14
      inhibiting activity in rats weighing approximately 200g
15
      and does not lead to the death of the rat.
16
17
      CGE-1 was found to contain Uscharin.
18
19
20
      EXAMPLE 2
21
      Isolation of Uscharin from Calotropis Gigantea leaves.
22
23
24
      EXTRACTION
25
26
      The plant material was minced to a fine powder in a
27
      bench grinder. The powder was extracted in a Soxhlet
      with petroleum ether (60-80) and the ethyl acetate,
28
      until exhaustion. The ethyl acetate fraction was
29
30
      concentrated to dryness using a rotary evaporator.
31
32
      FRACTIONATION
33
      Vacuum Liquid Chromatography was used for the initial
34
      fractionation of the crude extract Silica gel 60H
35
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(Merck) was packed in a scintered funnel under vacuum 1 to give a compact column. The crude extract, adsorbed 2 in silica, was applied to the column. Elution was initiated with petroleum ether and proceeded with 4 5 petroleum ether containing progressively greater amounts of ethyl acetate than with ethyl acetate 6 through to methanol. The fractions were concentrated 8 using a rotary evaporator. 10 mg of each fraction were... prepared for cytotoxicity testing (see MTT assay for 9 method) by solubilisation in DMSO. The fraction 10 containing the greatest cytotoxic activity was 11 subjected to a sephadex column to remove any remaining 12 13 chlorophyll. 14 15 SEPHADEX COLUMN The fraction was dissolved in a minimum volume of 17

16

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chloroform and applied to a column containing lipophilic sephadex LH-20 (Sigma) which had been packed in chloroform. Elution was with chloroform, chloroform with methanol and methanol. As before fraction were dried and tested for activity. The fraction with the greatest activity was further fractionated with a silica gel column.

25

26

### SILICA GEL COLUMN

27

The fraction was dissolved in a minimum volume of 28 chloroform and applied to a column containing silica 29 30 gel (packed in chloroform). Elution was with 31 chloroform, chloroform with methanol and methanol. 32 This column yielded a fraction of almost pure uscharin. 33 The pure compound was obtained from this fraction by 34 preparative TLC.

35

### PREPARATIVE TLC 1 2 The fraction was spotted onto glass silica gel plates. 3 The plates were run in ethyl acetate and methanol 4 (97:3). The silica was scratched from the plate and 5 the uscharin eluted with ethyl acetate. 6 Once the compound had been isolated, its identity was 8 confirmed by spectroscopic techniques. 9 10 EXAMPLE 3 11 12 CYTOTOXICITY BIOASSAY OF USCHARIN 13 14 Cytotoxicity bioassays were performed. The cell line 15 used was a human ovarian small cell carcinoma SCC Wm 16 1(151) which was grown as a monolayer in Dulbecco's 17 Modified Eagles Medium (Gibco) supplemented with 5% 18 foetal calf serum (v/v), sodium pyruvate (lmM), 19 penicillin (50IU/ml) and streptomycin (50 $\mu$ g/ml). 20 Cultures were maintained in a humidified atmosphere of 21 5% CO<sub>2</sub>/95% air at 37°. 22 23 Single cell suspensions were obtained by trypsinisation 24 of the monolayer cultures and an equal number of cells 25 (103-104 depending on the cell line) was inoculated into 26 each 33mm<sup>2</sup> well of a 96 well plate in 190µl of culture 27 medium. The plates were incubated for 24 hours to 28 allow cells to adhere. At this point 10µl of an 29 appropriate concentration of plant extract or control 30 solvent was added to each well. The cells were exposed 31 to the drug for 3 days after which the medium was 32 removed, the monolayers washed with PBS and fresh 33 medium added. This was repeated 24 hours later. 34

Following a further 24 hours incubation 100µg (50µl of

No. 11, (5th June, 1991).

2mg/ml in PBS) MTT (3-(4,5 dimethylthiazol-2-yl)-2, 5-1 diphenyltetrazolium bromide) was added to each well and 2 the cells were incubated at 37°C for 4 hours. Plates 3 were then processed using a modified version 4 (Carmichael et al, 1987) of the assay first described 5 by Mossman, T.(1983), where DMSO was used in preference 6 to acid isopropanol to solubilise the formazan 8 crystals. The contents of each well were mixed and the plate was read immediately at 540nm on a Flow Titertek 9 Multiscan MCC/340 Mk 11 plate reader. Cells were set 10 up in parallel at two densities,  $10^3$  and  $2 \times 10^3$ 11 12 cells/well, and the results from an assay were discarded if the ratio of the OD readings of the two 13 14 densities was greater than 2.25:1 or less than 1.75:1. 15 16 The • results obtained were as shown in Fig. 1 17 18 EXAMPLE 4 19 20 IN VITRO SCREENING OF USCHARIN 21 Uscharin was obtained as in Example 2 and was subjected 22 23 to in vitro cell screening at the National Cancer 24 Institute (NCI), USA in respect of a panel of cancel 25 cell types organised into subpanels representing 26 leukemia, lung cancers, colon cancer, cancer of the 27 central nervous system, melanoma, ovarian cancer, renal 28 cancer, and in some cases prostate cancer and breast 29 cancer also. 30 The standard NCI methodology which was employed is 31 32 described in Michael R Boyd, Principles and Practices 33 of Oncology, Vol. 3, No. 10 (Oct. 1989) and Monks A. et 34 al., Journal of the National Cancer Institute, Vol. 83,

```
The results of two separate screening experiements
1
      carried out using uscharin are given in Tables 1 and 2.
2
3
      The data are derived from Dose-Response Curves and two
4
      typical curves for leukemia and colon cancer are given
5
      for illustrative purposes in Figures 1 and 2 attached
6
      hereto.
7
8
      The Dose-Response Curve is created by plotting the
 9
      Calculated Percent Growth (PG) of each cell line
10
      against the log(10) of the corresponding drug
11
      concentration. The cell line curves are grouped by
12
      cell type, or subpanel. Mean Log(10) concentrations for
13
      all cell lines tested are calculated at three points:
14
      where the test compound achieved 50% inhibition of cell
15
      growth (GI50), where the test compound achieved 0% cell
16
      growth or total growth inhibition (TGI), and where the
17
      test compound achieved 50% cell kill or 50% lethal
18
      concentration (LC_{50}). Reference lines are shown at the
19
      percent growth values of +50 (GI<sub>50</sub>), 0 (TGI) and -50
20
21
      (LC_{50}).
22
      Percentage Growth (PG) - of the compound on a cell line
23
      is currently calculated according to one of the
24
      following expressions:
25
26
      If (Mean OD(test) - Mean OD(tzero) >= 0, then
27
28
      PG = 100 x (Mean OD(test) - Mean OD(tzero)/(mean
29
      OD(ctrl) - Mean OD(tzero)
30
31
       If (Mean OD(test - Mean OD(tzero) < 0, then PG = 100 x
32
       (Mean OD(test) - Mean OD(tzero)/Mean OD(tzero)
33 -
34
35
```

Where: 1 2 Mean OD (tzero) = The average of optical density 3 measurements of SRB-derived colour 4 just before exposure of cells to 5 6 the test compound. 7 8 Mean OD (test) = The average of optical density 9 measurements of SRB-derived colour 10 after 48 hours with no exposure of 11 cells to the test compound. 12 Mean OD (ctrl) = 13 The average of optical density 14 measurements of SRB-derived colour 15 after 48 hours with no exposure of 16 cells to the test compound. 17 18 It is clear from the results given in Tables 1 and 2 19 that uscharin has an inhibitory effect on the growth of 20 a wide variety of cancer cell lines in vitro. 21 22 EXAMPLE 5 23 24 IN VITRO SCREENING OF USCHARIDIN 25 26 Uscharidin was also subjected to in vitro cell 27 screening in the manner described in Example 4. 28 Results are given in Table 3 and Figure 3, and these 29 show that Uscharidin also exerts an inhibitory effect 30 on a variety of cancer cell lines in vitro. 31

EXAMPLE 6 1 2 3 IN VITRO SCREENING OF CALOTOXIN 4 Calotoxin was also subjected to in vitro cell screening 5 in the manner described in Example 4. Results are 6 given in Table 4 and Figure 4, which show that 7 calotoxin also exerts an inhibitory effect on a variety. 8 of cancer cell lines in vitro. 10 11 EXAMPLE 7 12 13 IN VITRO EXPERIEMENT WITH USCHARIN IN NUDE MICE 14 The SCCI cells (human tumour cell line) where grown (1 15 x 10<sup>5</sup>/ml seeding density) in 25 ml RPMI 1640 (10% foetal 16 calf serum, 5% glutamine) in 75 cm2 tissue culture 17 The cells were harvested at log growth phase 18 flasks. (5 days approximately) and washed once in saline before 19 20 injection into the mice. 21 22 The "nude" mice (BALB/c nude) are reared and contained within a sealed isolator. The mice were injected with 23 24 1 x 10<sup>7</sup> cells subcut on the back, right hand side near 25 the shoulder blades. After 7 days the mice were split 26 randomly into the study groups (10-15 animals per 27 group). Each was then treated with a different regime, 28 the variable being time between injections and dose of 29 drug at each injection, control groups were also 30 included in the overall plan of the experiement. 31 32 During the trial a daily check was made on the animals 33 and any animal removed if the tumour size became too 34 large (>5-7% total body weight) or if the animal is 35 showing signs of distress. Additional to this the

tumour should be assessed every 3-4 days by an independent observer and the result recorded. Once an animal is removed from the study the tumour size, volume and weight was determined and the tumour stored for further cytological study. The reason for the animals removal from the study was also recorded, if this was not due to tumour size. The results are shown in the following tables.

Using nude mice injected with  $10^7$  SCC-1 cells injected on day 0 and drug treatment started on day 9.

GROUP NO. 1
0.1 mg CGE-1/ Animal/ 5 days

			TU	MOUR		
MOUSE	DAY	VOL.	WEIGHT	RATE (mg/D)	NECROTIC	REASON
A	27	4356.4	1.7492	64.8	22.41	1
В	55	_	NONE	_	-	5
С	30	4141.3	2.5658	85.5	45.28	1
D	30	299.8	1.8196	60.7	52.24	1
Е	37	2752.8	1.5783	42.7	33.37	1
F	55		NONE	_	-	5
G	55		NONE	-	-	5
Н	55	_	NONE	-	_	5
I	33	3414.9	1.8805	57.0	28.69	1
J	55	<b>-</b>	NONE	-	-	5
K	37 :	828.9	0.6773	18.3	8.19	2
L	27	2223.8	1.6854	62.4	48.92	1
M	27	1556.2	0.7728	28.6	5.45	1
N	27	3457.9	1.9394	71.8	52.94	1
0	55	<b></b>	NONE	-	_	5
MEAN		2559.11	1.6298	54.64	33.05	
S.D.		1437.34	0.5844	21.20	18.29	

GROUP NO. 2

## 0.1 mg CGE-1/ Animal/ 10 days

			TU	MOUR	•	
MOUSE	DAY REMOVED	VOL.	WEIGHT	RATE (mg/D)	NECROTIC	REASON
A	27	2993.1	2.0570	76.2	49.92	1
В	55	-	NONE	1	_	5
С	55	-	NONE	_	-	5
D	55	-	NONE	_	-	5
В	55	664.8	0.4333	7.9	17.91	5
F	55	3148.8	2.0378	37.1	16.96	5
G	55	134.4	0.1285	2.3	8.17	5
Н	55		NONE	-		5
I	55	-	NONE	_	-	5
J	55		NONE	-		5
K	55	-	NONE	-	-	5
L	55	-	NONE	-	-	5
М	26	2025.9	1.3238	50.9	6.90	3
N	30	1548.8	1.2677	42.3	10.79	1
0	30	544.1	0.3827	12.8	25.29	4
MEAN		1579.99	1.0901	32.79	19.42	
S.D.		1201.27	0.7933	26.68	14.9	

GROUP NO. 3

0.5 mg CGE-1/ Animal/ 5 days

			, TU	MOUR		
MOUSE	DAY REMOVED	VOL. (mm³)	WEIGHT	RATE (mg/D)	NECROTIC (%)	REASON
A	55	-	NONE		_	5
В	55	219.6	0.2082	3.8	18.18	5
С	55	_	NONE	-	-	5
D	19	1494.7	1.1889	62.6	2.33	3
	19	203.2	0.0948	5.0	-	
E	19	_	NONE	-		3
F	23	3912.0	2.5341	110.2	13.13	1
G	28	4463.2	2.5717	91.8	23.42	1
Н	37	-	NONE	-	_	2
I	28	1666.5	1.0930	39.0	12.96	1
J	19	23.7	0.0038	0.2	-	3
K	33	1457.9	1.2546	38.0	19.22	1
L	29	1532.5	0.8926	30.8	12.49	1
М	29	2972.3	1.6348	56.4	17.79	1
N	37	537.9	0.4997	13.5	9.70	2
0	37	-	NONE		_	2
MEAN		1848.36	1.1976	45.12	14.36	
S.D.		1504.32	0.8738	36.61	6.18	

GROUP NO. 4

0.5 mg CGE-1/ Animal/ 10 days

			TU	MOUR		
MOUSE	DAY	VOL.	WEIGHT	RATE (mg/D)	NECROTIC (%)	REASON
A	28	1482.1	1.1211	40.0	28.48	1
В	27	3499.1	2.5087	92.9	32.54	1
С	42	1930.3	1.4088	33.5	13.58	1
D	42	2177.3	1.5067	35.9	17.14	1
E	55	_	NONE	-	_	5
F	27	6882.3	3.1626	117.1	42.37	1
G	33	760.9	0.7467	22.6	50.31	1.
H.	55	_	NONE	-	-	5
I	55	_	NONE	_	-	5
J	55	_	NONE	-	_	5
K	55	64.5	0.1127	2.0	17.78	5
L	29	_	NONE	-	-	2
М	55	-	NONE	<u>-</u>	_	5
N	23	4929.6	2.6126	113.6	37.52	1
0	55	-	NONE	· <b>-</b>	_	5
MEAN		2715.76	1.6475	57.2	29.97	
S.D.		2272.64	1.0344	44.08	13.18	

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GROUP NO. 5
CONTROL (0.1 ml Saline/ Animal/ 5 days

			TUM	10UR		
10USE	DAY	VOL. (mm³)	WEIGHT	RATE (mg/D)	NECROTIC (%)	REASON
A	55	-	NONE		-	5
 В	55	_	NONE	-	-	5
	55	-	NONE	_	_	5
 D	55	-	NONE	-		5
 E	23	4570.9	2.4227	105.3	35.2	1
 F	50	3138.3	1.9475	39.0	4.43	1
G	55	-	NONE	-	-	5
Н	55	-	NONE	-		5
I	3	-	NONE	-	-	3
J	23	5493.0	3.1602	137.4	59.07	1
K	28	2500.7	1.8958	67.7	6.68	1
L	28	3246.9	1.9716	70.4	31.86	1
M	55	- :	NONE			5
N	28	4120.3	2.2965	82.0	46.07	1
0	55		NONE	_	-	5
MEAN		3845.02	2.270	7 83.63	30.55	
S.D.		1093.88	0.479	7 34.01	21.59	

# 1 NOTES:-**REASONS:**

- Removed due to tumour size. (1)
- Removed due to another illness. (2)
- Found dead in cage. (3)
- (4) Removed because the tumour was about to rupture.
- Removed at end of the experiment. (5)

TABLE 5 Table 5 gives a summary of the results.

•	Tumour Growth (mg/day)	% Necrosis*	% Mortality at 40 days
Group 1 (0.1mg/5 days)	54.6 ± 21.1	33.1 ± 18.3	84
Group 2 (0.1mg/10 days)	32.8 ± 26.7	19.4 ± 14.9	55
Group 3 (0.5mg/5 days)	45.1 ± 36.6	14.4 ± 6.2	90
Group 4 (0.5mg/10 days)	57.2 ± 44.1	30.0 ± 13.2	62 ·
Control	83.6 ± 34.0	30.6 ± 21.6	100

\* from histological examination Values are means ±SD, n=15

From these results it can be seen that a reduction in percentage mortallity due to the cancer cells of up to 45% can be achieved by administration of the compound of the invention (Uscharin).

### CLAIMS

2

1

1. A composition comprising uscharin or analogues or salts thereof as active ingredient together with a pharmaceutically acceptable carrier or excipient.

6

7 2. The use of uscharin, analogues or salts thereof 8 for medical (including veterinary) purposes.

9

10 3. The use of uscharin as claimed in the preparation of a medicament.

12

13 4. A composition as claimed in Claim 1 or 2 wherein 14 the uscharin is suspended or dissolved in an 15 acceptable liquid carrier medium.

16

17 5. A composition as claimed in Claim 4 wherein the carrier medium is aqueous based.

19

20 6. A use as claimed in Claims 2 or 3 wherein 0.1-100 uscharin per kg body weight is used.

22

7. A method of treatment of a human or non-human animal body, said method comprising administering to said body a composition comprising uscharin.

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27 8. A method as claimed in Claim 7 wherein a unit dose 28 of composition comprises between 20 and 500 mg 29 uscharin.

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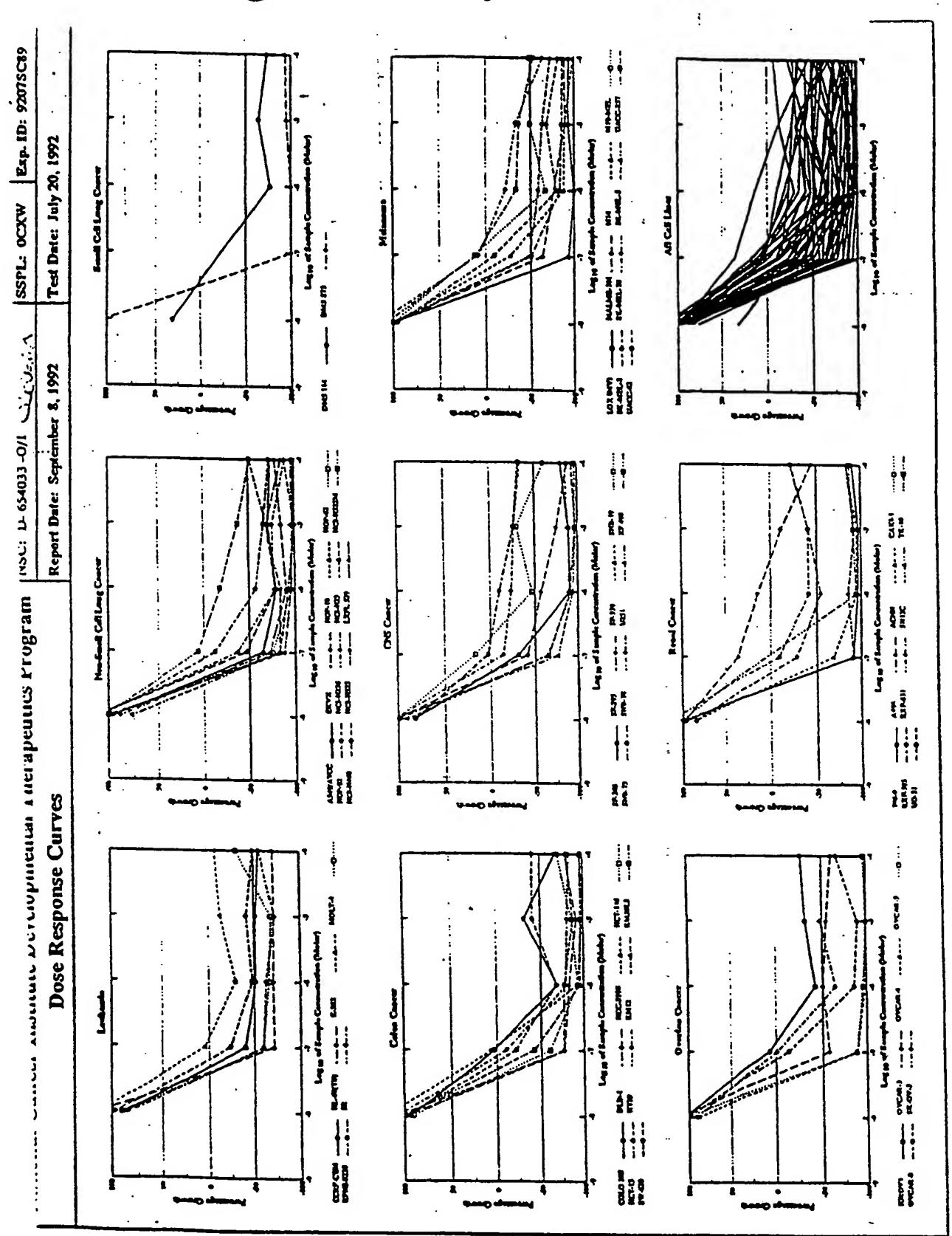
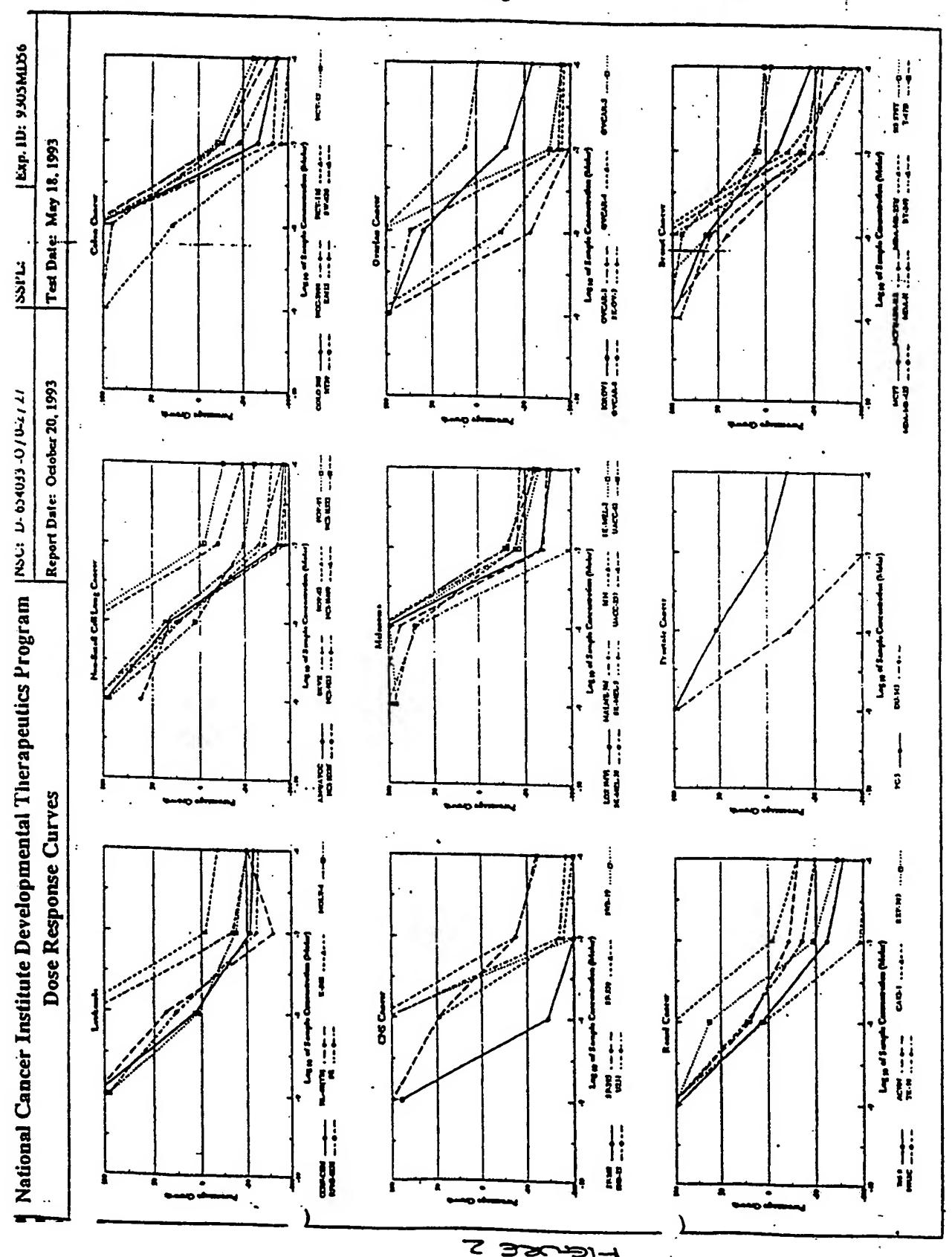


FIGURE I

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K-362 NOLT-4	0.120	0.823	0.804		0.045	0.104	0.111	111	4 •	-30 -	13	-7	3.74E-08 1.83E-08	1.358-07 >	1.002-04	••
RPH2-8226	0.345	1.374	1.350	0.414	0.284	0.314	0.274	57	-24 -	-46 -	42 -	49	2.438-00	4.345-04 >	1.00E-04	
SR Mon-Small Coll L		1.430	1.279	0.138	0.127	9.094	0.150	84	-60 -	-44 -	-73 -	57	1.732-04	1.435-04	4.462-06	
AS49/ATCC	0.381	1.657	1.393	0.133	0.076	0.109	0.094					74	1.91E-08		8.642-04	
NOS –7 8 EKAX	•		•	•	•	0.353	•	•	•	•	•	•	1.432-06	•	1.282-07	
HOP-62 HOP-92	0.434	1.702		0.208	0.015		0.014	100				73	1.921-06		7.128-06 6.755-07	
HCI-H226	0.519	1.325	1.367	0.572	0.199	8.220	0.093	110	-34	-78 -	-76 -	90	2.368-06	1.37E-08	2.01E-07	
MCI = N23 MCI = N322H		1.407	1.201	0.087	0.080	0.157	0.250	77 104				32 32	1.47E-08 3.57E-08		4.21E-08 7.60E-05	
HCI -H4 60	0.177	1.224	1.151	0.030	0.013	-0.002	0.614	94	-83	-93 -	100 -	90	1.802-04	3.43E-06	6.32E-04	
NCI-N322 LXFL 329		0.763		0.130	0.044		0.096					-80 -97	1.73K-08 1.86K-08	J.335-06 J.416-06	7.23E-01 6.27E-01	
Small Call Lung DMS 114	Cancer			0 204	0.300	0.158	0.116	31		-77	-44 -	-74	<1.00E-08			
DHS 273					-0.012	0.013	0.016		100 -		-	-94		3.188-04	3.12E-07 3.64E-08	
Colon Cander COLO 203	0 277	1 284	1.215	0.300	0.047	0.188	0.051	93	2	-69	-12	-67	2.988-08	1.065-07		
DLD-1	0.153	0.444	0.844	0.035	0.026	0.012	0.030	97	-77	-03	-12	-00	1.862-08	3.392-08	6.96E-04	
NCC-2998 HCT-114			0.906		0.022	0.004	0.010	110				-97 -71		1.118-07		
HCT-15	0.312	1.790	1.881	0.073	0.072	0.037	0.060	106	-77	-77	-44	-01	2.03E-08	3.415-04	7.162-01	
NT29 NG12	•	•	1.342	•	•	•	•	107	•	•	-62	-14	•	8.032-08	•	
10(2012 \$W+620						0.008		101	-43		-97 -41		2.27E-08 2.31E-08	5.03E-08	1.385-07	
QIS Cander	•														•	
57-268 57-295						0.045	0.093		-32 -42		-90 -76			\$.272-06 \$.12E-08		
\$F-539	0.946	1.793	1.702	0.304	0.06:	6.693	0.113	90	-64	-93	-19	-67	1.832-06	3.858-08	8.11E-06	
818-19 818-73		0.864	0.411		0.303		0.337	107	16	-10		-61 -33		1.81E-07 1.60E-07		
5XB-74 U251		1.091				0.405					-27 -96		2.862-08	7,502-08	>1.005-04	
XF 498							0.013.				-97		1.982-08	3.78E-08 3.99E-06	1.11E-04 1.04E-04	
Heisnems LOX INVI	0.254	1.149	1.144	0.014	-0.001	0.017	0.014	41	-94	-100	-94	-94		1.232-01		
KALINE-3M	0.644	1.259	1.253	0,233	0.124	0.066	0.069	99	-64	•	-90	-47	2.012-08	4.04E-06	1.26L-01	
nl4 nl9-nel	0.284	1.126	1.124	0.316	0.054	0.033	0.146	100		-67	-49	-99 -49	3.698-01	4.03E-06 1.42E-07		
8K-XXL-2 8K-XXL-28	0.370	1.357	1.322	9,240	0.097	0.070	0.073	115	-51	-44	-86 -33		2.052-08	4.30E-00 1.80E-07		
SK-MEL-5	0.445	1.905	1.056	0.241	0.200	0.179	0.134	**	-49	-59	-63	-72	2.16E-08	4.49E-04	1.365-07	
UACC-237 UACC-62			2,117			3 0.463 3 0.163	0.344	106			-37 -64	-53 -82	1.86E-08	1.72E-07 1.04E-04	4.40E-05	
Ovatian Cancer																
IGROV1 OVCAR-3			1.422	9.28	0.32	4 0.296	0.320	105			-32 -35		2.278-06	4.532-08	>1.00E-04 7.03E-06	
OVEAR-4 OVEAR-5	8,417	1.031	0.974	0.04	0.01	7 -0.00	0.004	101	-11		-99 -100	-99 -97	1.645-08	3.138-08 3.428-08	6.03E-08	
OVCAR-8	0.61	1.764	1.799	0.33	0.09	2 0.045	0.206	101	-14	-43	-90		2.79E-00	7.402-08	3.22E-07	
SK-OV-1 - Renal Cancer	0.469	3 1.149	1.097	0.48	0 0.17	2 0.25	0.122	90	1	-64	-46	•	2.735-00	9.728-08		
744-0							0.027		-90		-97		1.778-0	3.26E-06	4.092-04	
A496 ACIUI			1.340				0.341		34 3 -48		-12 -95		3.46%-01 1.68%-01	J.342-04 J.372-01	>1.00E-04 7.38E-08	
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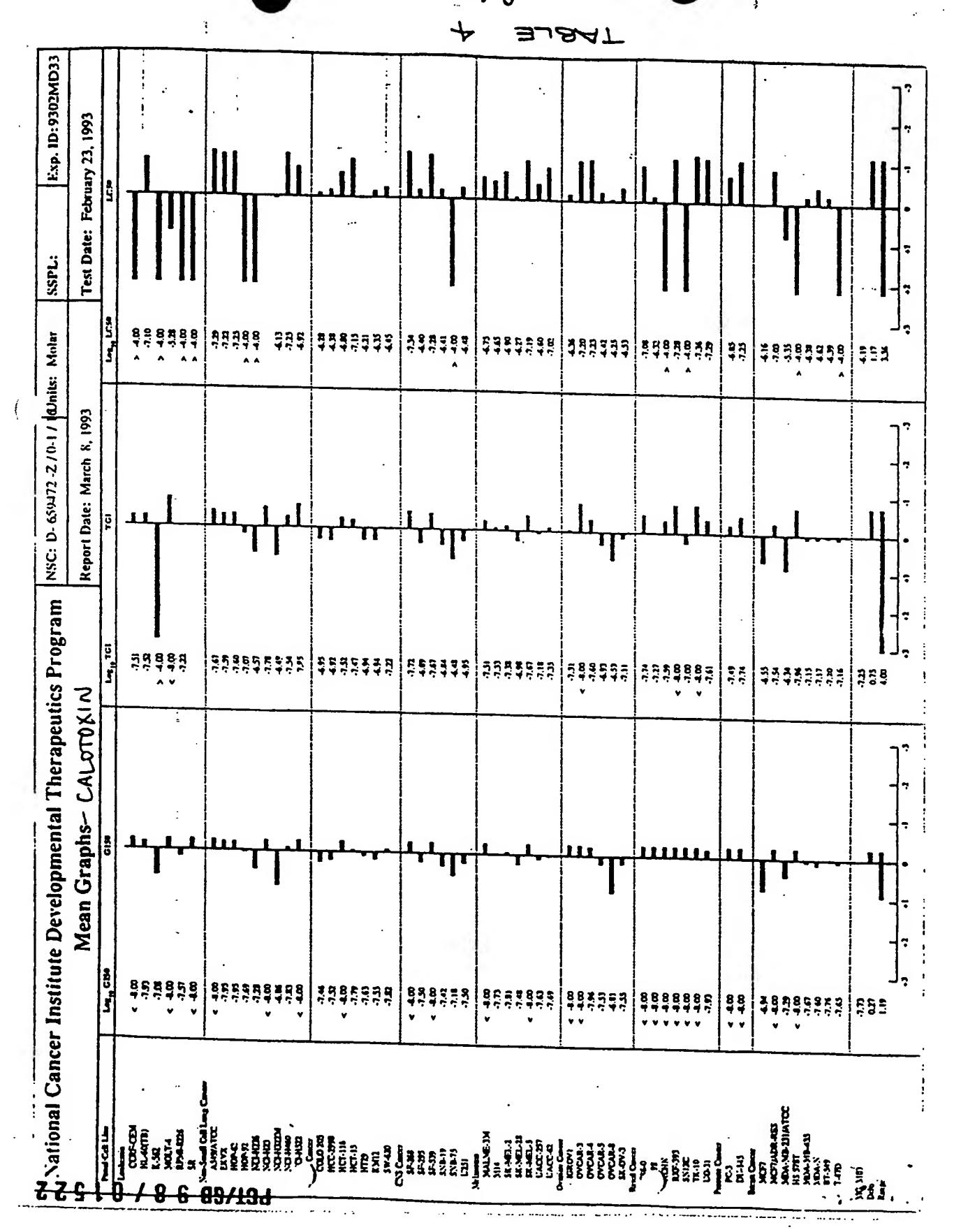
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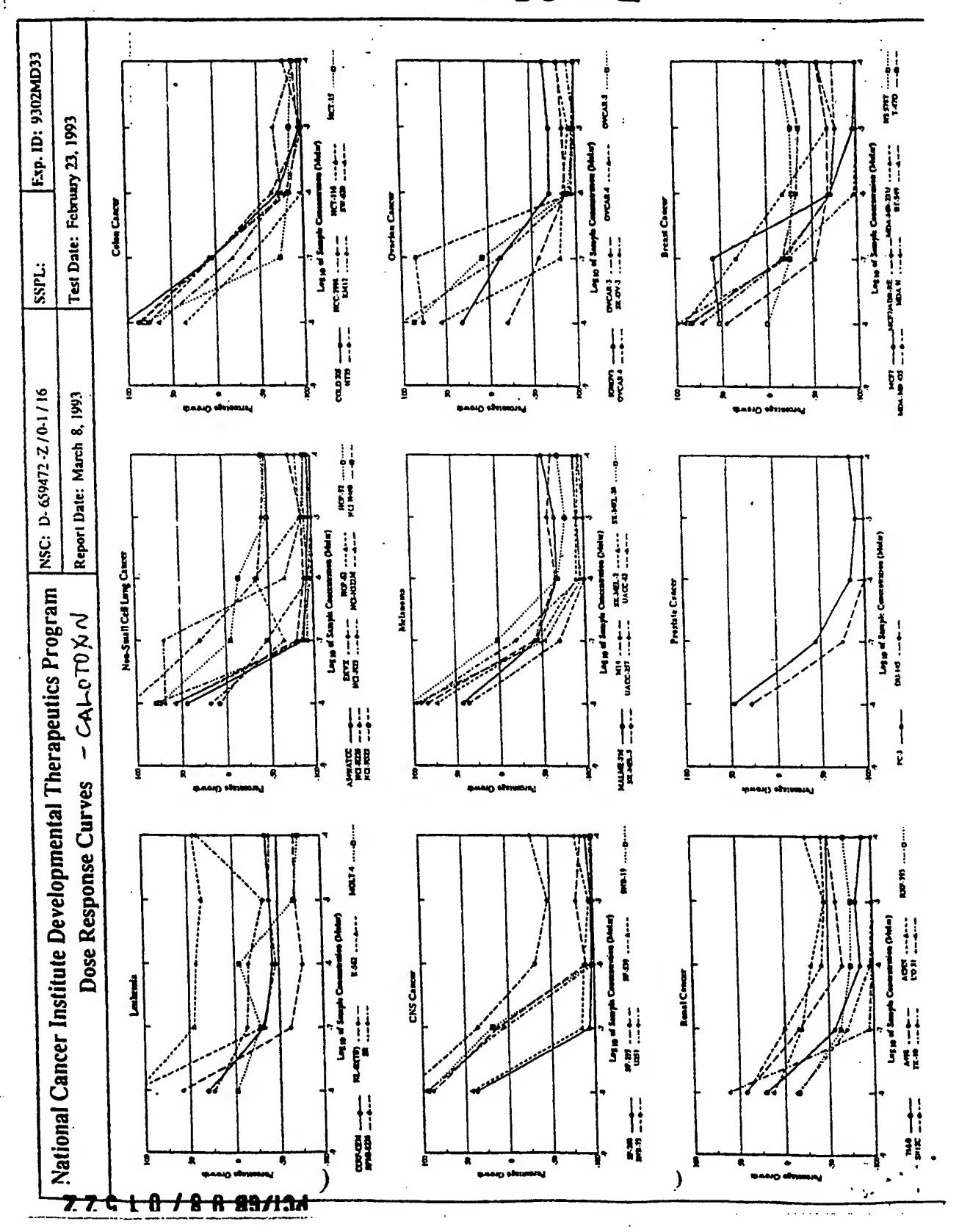


E 31847 Exp. 1D: 9302MD33 February 23, 1993 Test Date: SSPL: NSC: D. 659471 -Y / 0-1 / Dults: Molar 684444 Report Date: March 8, 1993 A National Cancer Institute Developmental Therapeutics Program - USCHARIDIN Mean Graphs

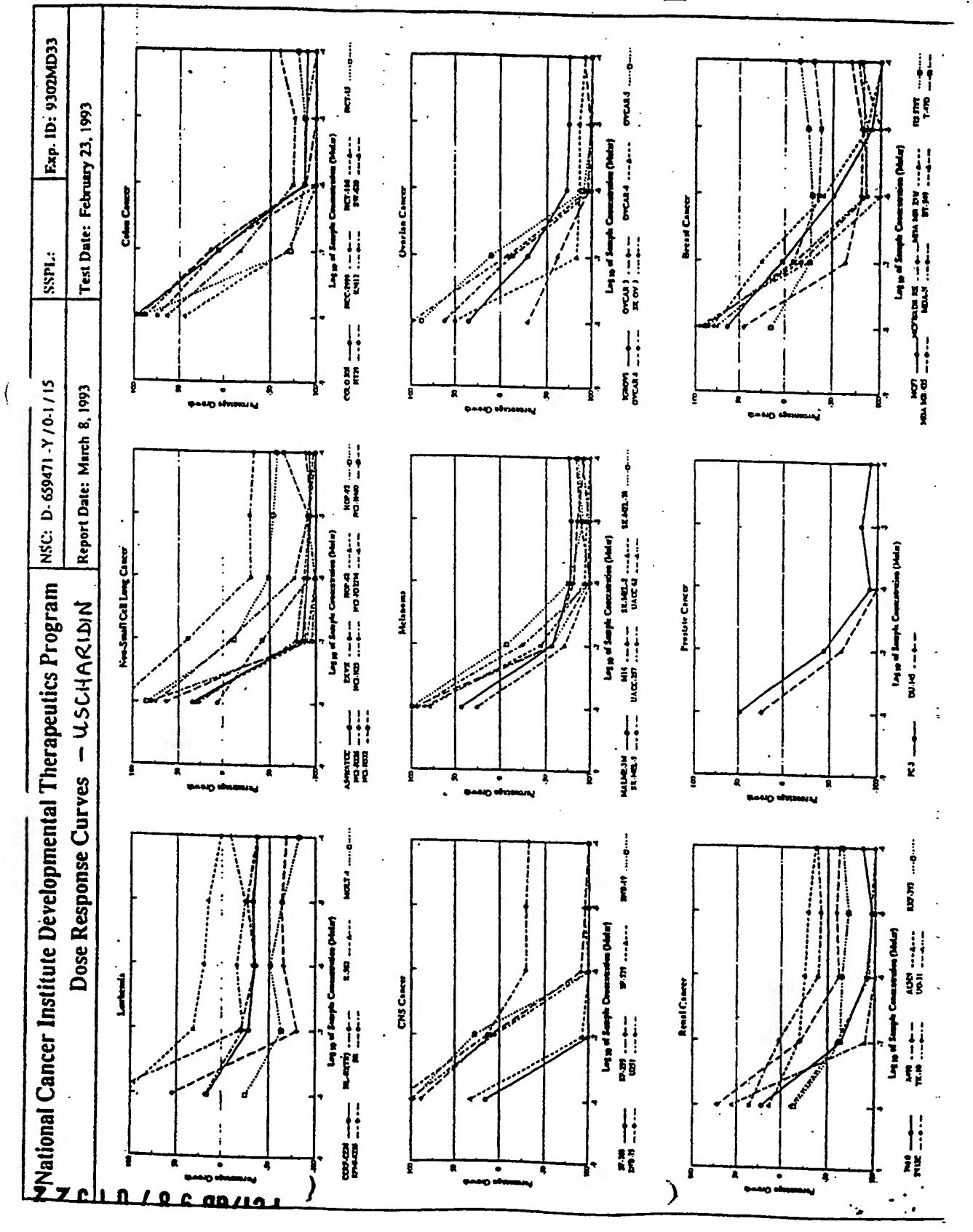
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FIGUSE



# INTERNATIONAL SEARCH REPORT

Int Honal Application No

			PCT/GP 201522 -
A. CLASSIF	ICATION OF SUBJECT MATILEN A61K31/365		÷ .
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ccording to	International Patent Classification (IPC) or to both national classifi	cation and IPC	
	SEARCHED		•
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Documentati	ion searched other than minimum documentation to the extent that	such documents are inclu	ided in the fields searched
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0.000	ENTS CONSIDERED TO BE RELEVANT		
Category °	Citation of document, with indication, where appropriate, of the	relevant passages	Relevant to claim No.
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	action of digitalis and elated (digitoxin, digoxin, lanatoside	glycosides C quabain	
	and calactin)"	. 0 04424777	
	BR. J. PHARMACOL.,	152	
	vol. 42, no. 1, 1971, pages 143 XP002078318		
	see page 145		
Р,Х	F. KIUCHI ET AL.: "Cytotoxic I	oriciples of	1-6
ι , Λ	a Bangladesh crude drug, akond		
	of Calotropis gigantea L.)"		
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	XP002078319	·	
	see the whole document		
Α	WO 92 09295 A (MRAK, M.,) 11 J	une 1992	-
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	tion) DOCUMENTS CONRED TO BE RELEVANT	
Category 3	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	A.E.MUTLIB ET AL.: "In vivo and in vitro metabolism of gomphoside, a cardiotonic steroid with doubly-linked sugar."  J. STEROID BIOCHEM., vol. 28, no. 1, 1987, pages 65-76, XP002078320	
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information on patent family members

Int. tional Application No
PCT/GB 01522 —

Patent document cited in search report		Publication date	· ·	atent family member(s)	Publication date
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(74) Agent: MURGITROYD & COMPANY; 373 Scotland Street, Glasgow G5 8QA (GB).

(81) Designated States: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, GH, GM, GW, HU, ID, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG).

#### **Published**

With international search report.

Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.

(54) Title: PHARMACEUTICAL COMPOSITION CONTAINING USCHARIDIN OR ITS ANALOGUES

### (57) Abstract

The invention provides compositions comprising uscharin and the use of uscharin to combat cell proliferation for example in the treatment of cancer. Administration of uscharin may kill or reduce the growth rate of cancer cells and may also be of application in other medical conditions presenting symptoms of excessive or uncontrolled cell proliferation. The composition may be administered by any convenient route and formulated accordingly. The composition may be administered locally or generally and may be suitably dissolved and/or suspended in a pharmaceutically acceptable liquid carrier medium.

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CZ	Czech Republic	LC	Saint Lucia	RU	Russian Federation		
DE	Germany	LI	Liechtenstein	SD	Sudan		
DK	Denmark	LK	Sri Lanka	SE	Sweden		
EE	Estonia	LR	Liberia	SG	Singapore		

PHARMACEUTICAL COMPOSITION CONTAINING USCHARIDIN OR ITS ANALOGUES This invention relates to a composition comprising the cardenolide glycoside uscharin. Plants of the family Asclepidaceae are known to be extremely poisonous. Such plants have a history of use in folk medicines in those areas where they occur naturally, for example in South East Asia and Africa. 10 Two of the best known representatives of the Asclepiadaceae are Calotropis gigantea and Calotropis 11 procera. Extracts from Calotropis procera plants have 12 traditionally been used as an abortifacient, for 13 14 infanticide, for rheumatic pain and to produce a 15 purgative. 16 17 The stems, flowers and leaves of plants from the family 18 Asclepiadaceae (including Calotropis gigantea and 19 Calotropis procera) are known to contain certain 20 compounds known as cardenolides. In several species 21 substantial amounts of cardenolides have been found to 22 be concentrated in the latex (Roeske et al, in 23 Biochemical Interactions Between Plants and Insects

published in Volume 10 of Recent Advances in

1 Phytochemistry, Plenum Press, New York (ed. Wallace), 2 Seiber et al, Phytochemistry 21:2343 (1982), Seiber et al, in Isopentoids in Plants, Academic Press (ed Nes, 1984) and Seiber et al, in J. Chem. Ecol. 6:321 4 5 (1980)). The natural production of cardenolides in 6 Ascelopias curassavia has been reported by Groeneveld et al in Phytochemistry 29(11):3479-3486 (1990). Examples of cardenolide glycosides found in C. procera 8 are voruscharin, uscharin, uscharidin, calotropin, 10 calactin, calotoxin, and calotropagenin. Formula I 11 shows the chemical structure of these cardenolides. 12

It has now been found that the cardenolide uscharin is 1 2 particularly useful for medical purposes. Whilst uscharin has been isolated and its chemical structure determined, no utility for this compound has previously 4 5 been reported. 6 7 The present invention thus provides a composition 8 comprising uscharin, the analogues and salts thereof as 9 active ingredient together with a pharmaceutically 10 acceptable carrier or excipient. 11 12 Further, the present invention also provides the use of 13 uscharin, the analogues and salts thereof for medical 14 (including veterinary) purposes. 15 16 Previously, certain cardenolide glycosides such as 17 calotropin and uzarigenin have been noted to have 18 cytotoxic activity against primate tumour cells. 19 Certain cardenolide glycosides from the Asclepiadaceae 20 family share structural and pharmacological 21 similarities with the Digitalis cardiac glycosides. 22 Whilst we do not wish to be bound by theoretical 23 considerations it is believed that the cytotoxicity of 24 some cardenolide glycosides is related to the 25 inhibition of the plasma membrane bound Na<sup>+</sup>/K<sup>+</sup> ATPase 26 (ie analogous to the manner in which Digitalis cardiac 27 glycosides exert their toxic effects). However, it has 28 also been shown that whilst some cardenolide glycosides 29 are cytotoxic to cell cultures they have no in vivo 30 tumour-inhibiting activity. This is true of calotropin 31 and uzarigenin. 32 33 It has never previously been proposed that uscharin 34 would be useful for medical applications. The inventors' results have shown that at lmg/ml a primary 35

extract of Calotropis gigantea known as CGE-1 does have 1 tumour inhibiting activity in rats (weighing about 2 200g) and does not lead to the death of the test animals. 4 5 Typically, the use of uscharin according to the present 6 invention is to combat cell proliferation for example 7 in the treatment of cancer. Thus administration of 8 uscharin may kill or reduce the growth rate of cancer 9 cells and may also be of application in other medical 10 conditions presenting symptoms of excessive or 11 uncontrolled cell proliferation. 12 13 The word "combat" is used herein to refer to treatment 14 of an existing condition so as to alleviate or reverse 15 the symptoms of the condition in an affected human or 16 animal and to prevent such a condition in a healthy 17 18 human or animal. 19 The composition according to the present invention may 20 be administered by any convenient route and mention may 21 be made of enteral, parenteral, topical administration 22 and the composition will be formulated accordingly. 23 Conveniently, the composition may be administered 24 locally to the affected site, generally by means of 25 injection. Thus the uscharin will be suitably 26 dissolved and/or suspended in a pharmaceutically 27 acceptable liquid carrier medium, which will generally 28 be aqueous-based, for example an isotonic solution. 29 Alternatively, the composition according to the 30 invention may be taken orally. 31 32 Formulations for parenteral administration include 33 aqueous and non-aqueous isotonic sterile injection 34 solutions which may contain anti-oxidants, buffers, 35

bacteriostats and solutes which render the formulation 1 isotonic with the blood of the intended recipient; and 2 aqueous and non-aqueous sterile suspensions which may 3 include suspending agents and thickening agents. formulations may be presented in unit-dose or multi-5 dose sealed containers, for example, ampoules and 6 7 vials, and may be stored in a freeze-dried (lyophilized) condition requiring only the addition of 8 the sterile liquid carrier, for example water for 9 10 injections, immediately prior to use. Extemoraneous injection solutions and suspensions may be prepared 11 from sterile powders, granules and tablets of the kind 12 13 previously described. 14 15 The dose will depend on a number of factors known to 16 the skilled physician including the severity of the conditions, the identity of the recipient; and also the 17 efficacy and toxicity of the particular composition 18 which is being administered. Generally doses in the 19 range 0.1-100 mg/kg body weight may be used, 20 particularly 1-10 mg/kg. The frequency of 21 22 administration will vary depending on the rate of 23 metabolism or excretion of the administered compound, 24 but may be repeated daily, optionally as two or more sub-doses. Unit doses of 20 to 500 mg, preferably 100 25 26 to 400 mg may be used. 27 28 A single dosage may be given daily or smaller 29 quantities or dosage units may be given at intervals 30 throughout a 24 hour period, for example dosage units 31 given 2, 3 or 4 times throughout the day. 32 Any type of cancer or condition involving cell 33 proliferation may be treated by the present invention. 34

Uscharin is especially useful for the treatment of

cancers such as leukaemia, non-small cell lung cancer, 1 small cell lung cancer, colon cancer, CNS cancer, melanoma, ovarian cancer, renal cancer, prostrate 3 cancer, and breast cancer. However the invention is not limited to treatment of these specific conditions 5 since uscharin is believed to be of general effect. 6 7 Cancers where uscharin is particularly efficacious 8 include ovarian cancer and skin cancer. 10 Uscharin may by produced by any convenient method, for 11 12 example by chemical synthesis. Alternatively the 13 uscharin may be conveniently extracted and purified 14 from organisms (for example plants of the family 15 Asclepiadacaeae) which produce uscharin naturally. It 16 is also envisaged that uscharin may be manufactured using genetically engineered micro-organisms, plants or 17 18 animals or may be made using cell-culture or other 19 biotechnological techniques. 20 21 Further, the present invention also provides the use of 22 a composition as described above for medical purposes, 23 for example to combat conditions in which cell 24 proliferation is undesirable (eg cancer). 25 26 In another aspect, the present invention provides the 27 use of uscharin in the manufacture of a medicament. 28 Generally such medicament would be of use to combat 29 cancer and other conditions where cell proliferation is 30 undesirable. 31 32 In a further aspect, the present invention provides a 33 method of treatment of a human or non-human animal 34 body, said method comprising administering to said body 35 a composition as described above.

The present invention is now further described by means of the following, non-limiting Examples. EXAMPLE 1 4 PREPARATION OF USCHARIN EXTRACT 6 7

ISOLATION OF CGE-1 (i)

8

Leaves of Calotropis gigantea (500g) were Soxhlet 10 extracted initially with petroleum ether (60-80), then 11 ethyl acetate and finally methanol. The cell culture 12 bioassays showed that the ethyl acetate fraction 13 contained cytotoxic activity. The ethyl acetate 14 extract was subjected to vacuum liquid chromatography 15 (VLC) on silica gel 60H (Merck). Elution was initiated 16 with petroleum ether (60-80) and proceeded with 17 petroleum ether containing progressively greater 18. amounts of ethyl acetate through to ethyl acetate only. 19 Elution was then continued with ethyl acetate 20 containing progressively greater amounts of methanol. 21

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Samples of the fraction were collected and prepared for cytotoxicity testing by solubilisation in 0.1% Tween.

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The greatest cytotoxic activity ( $ED_{50} < 0.10 \mu g/ml$ ) was found in the 70-80% ethyl acetate in petroleum ether fractions. The cytotoxic compound CGE-1 (72.0 mg)  $(ED_{50} < 0.09 \mu g/ml)$  was isolated as a white semicrystalline precipitate from this fraction.

31

#### 32 (ii) ISOLATION OF CGE-2

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34 Another less cytotoxic compound, CGE-2 (101.0mg) (ED<sub>50</sub> 35 <8.0µg/ml) was isolated from the 100% ethyl acetate

fraction as a semi-crystalline precipitate. 1 PROPERTIES OF CGE-1 (iii) 4 White powder, found 587.2511, C31H41NO8S requires 5 587,2553.  $[\alpha]_0 + 10.0^{\circ} (c.0.1, CH_3OH_4)$  IR 6  $V_{max}$  CM<sup>-1</sup>: 3465, 2960, 2920, 2840, 2720, 1735, 1730, 1705, 1625, 1540, 1160, 1110, 1060, 1040. EIMS m/z 8 (rel. int.) 587 [M+] (4.0), 233 (14.9), 215 (8.6), 187 9 (9.8), 18310 11 ACTIVITY OF CGE-1 12 13 At a concentration of 1 mg/ml, CGE-1 has a tumor 14 inhibiting activity in rats weighing approximately 200g 15 and does not lead to the death of the rat. 16 17 CGE-1 was found to contain Uscharin. 18 19 20 EXAMPLE 2 21 Isolation of Uscharin from Calotropis Gigantea leaves. 22 23 24 **EXTRACTION** 25 The plant material was minced to a fine powder in a 26 bench grinder. The powder was extracted in a Soxhlet 27 with petroleum ether (60-80) and the ethyl acetate, 28 until exhaustion. The ethyl acetate fraction was 29 concentrated to dryness using a rotary evaporator. 30 31 32 FRACTIONATION 33 Vacuum Liquid Chromatography was used for the initial 34 fractionation of the crude extract Silica gel 60H 35

(Merck) was packed in a scintered funnel under vacuum 1 to give a compact column. The crude extract, adsorbed 2 in silica, was applied to the column. Elution was initiated with petroleum ether and proceeded with 4 petroleum ether containing progressively greater 5 6 amounts of ethyl acetate than with ethyl acetate through to methanol. The fractions were concentrated 7 using a rotary evaporator. 10 mg of each fraction were 8 9 prepared for cytotoxicity testing (see MTT assay for method) by solubilisation in DMSO. The fraction 10 11 containing the greatest cytotoxic activity was 12 subjected to a sephadex column to remove any remaining 13 chlorophyll.

14

15

### SEPHADEX COLUMN

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17 The fraction was dissolved in a minimum volume of 18 chloroform and applied to a column containing 19 lipophilic sephadex LH-20 (Sigma) which had been packed in chloroform. Elution was with chloroform, chloroform 20 21 with methanol and methanol. As before fraction were dried and tested for activity. The fraction with the 22 23 greatest activity was further fractionated with a 24 silica gel column.

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### SILICA GEL COLUMN

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The fraction was dissolved in a minimum volume of 28 chloroform and applied to a column containing silica 29 30 gel (packed in chloroform). Elution was with 31 chloroform, chloroform with methanol and methanol. 32 This column yielded a fraction of almost pure uscharin. 33 The pure compound was obtained from this fraction by 34 preparative TLC.

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34

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1 PREPARATIVE TLC The fraction was spotted onto glass silica gel plates. The plates were run in ethyl acetate and methanol 4 (97:3). The silica was scratched from the plate and 5 6 the uscharin eluted with ethyl acetate. 7 8 Once the compound had been isolated, its identity was confirmed by spectroscopic techniques. 9 10 11 EXAMPLE 3 12 13 CYTOTOXICITY BIOASSAY OF USCHARIN 14 15 Cytotoxicity bioassays were performed. The cell line 16 used was a human ovarian small cell carcinoma SCC Wm 17 1(151) which was grown as a monolayer in Dulbecco's 18 Modified Eagles Medium (Gibco) supplemented with 5% 19 foetal calf serum (v/v), sodium pyruvate (1mM), 20 penicillin (50IU/ml) and streptomycin (50 $\mu$ g/ml). 21 Cultures were maintained in a humidified atmosphere of 22  $5\% CO_{2}/95\% air at 37°$ . 24 Single cell suspensions were obtained by trypsinisation 25 of the monolayer cultures and an equal number of cells 26 (103-104 depending on the cell line) was inoculated into 27 each 33mm<sup>2</sup> well of a 96 well plate in 190µl of culture 28 medium. The plates were incubated for 24 hours to 29 allow cells to adhere. At this point 10µl of an 30 appropriate concentration of plant extract or control 31 solvent was added to each well. The cells were exposed 32 to the drug for 3 days after which the medium was

removed, the monolayers washed with PBS and fresh

Following a further 24 hours incubation 100µg (50µl of

medium added. This was repeated 24 hours later.

No. 11, (5th June, 1991).

2mg/ml in PBS) MTT (3-(4,5 dimethylthiazol-2-yl)-2, 5-1 2 diphenyltetrazolium bromide) was added to each well and the cells were incubated at 37°C for 4 hours. Plates were then processed using a modified version 4 5 (Carmichael et al, 1987) of the assay first described by Mossman, T. (1983), where DMSO was used in preference 6 to acid isopropanol to solubilise the formazan 7 8 crystals. The contents of each well were mixed and the plate was read immediately at 540nm on a Flow Titertek 9 10 Multiscan MCC/340 Mk 11 plate reader. Cells were set 11 up in parallel at two densities,  $10^3$  and  $2 \times 10^3$ 12 cells/well, and the results from an assay were 13 discarded if the ratio of the OD readings of the two 14 densities was greater than 2.25:1 or less than 1.75:1. 15 16 The results obtained were as shown in Fig. 1 17 18 EXAMPLE 4 19 20 IN VITRO SCREENING OF USCHARIN 21 22 Uscharin was obtained as in Example 2 and was subjected 23 to in vitro cell screening at the National Cancer 24 Institute (NCI), USA in respect of a panel of cancel 25 cell types organised into subpanels representing 26 leukemia, lung cancers, colon cancer, cancer of the 27 central nervous system, melanoma, ovarian cancer, renal 28 cancer, and in some cases prostate cancer and breast 29 cancer also. 3.0 31 The standard NCI methodology which was employed is 32 described in Michael R Boyd, Principles and Practices 33 of Oncology, Vol. 3, No. 10 (Oct. 1989) and Monks A. et 34 al., Journal of the National Cancer Institute, Vol. 83,

```
The results of two separate screening experiements
 1
      carried out using uscharin are given in Tables 1 and 2.
      The data are derived from Dose-Response Curves and two
 4
      typical curves for leukemia and colon cancer are given
 5
      for illustrative purposes in Figures 1 and 2 attached
 6
      hereto.
 7
8
      The Dose-Response Curve is created by plotting the
      Calculated Percent Growth (PG) of each cell line
10
      against the log(10) of the corresponding drug
11
      concentration. The cell line curves are grouped by
12
      cell type, or subpanel. Mean Log(10) concentrations for
13
      all cell lines tested are calculated at three points:
14
      where the test compound achieved 50% inhibition of cell
15
      growth (GI<sub>50</sub>), where the test compound achieved 0% cell
16
      growth or total growth inhibition (TGI), and where the
17
      test compound achieved 50% cell kill or 50% lethal
18
      concentration (LC50). Reference lines are shown at the
19
      percent growth values of +50 (GI<sub>50</sub>), 0 (TGI) and -50
20
21
      (LC_{50}).
22
      Percentage Growth (PG) - of the compound on a cell line
23
24
      is currently calculated according to one of the
25
      following expressions:
26
      If (Mean OD(test) - Mean OD(tzero) >= 0, then
27
28
      PG = 100 \times (Mean OD(test) - Mean OD(tzero)/(mean)
29
30
      OD(ctrl) - Mean OD(tzero)
31
      If (Mean OD(test - Mean OD(tzero) < 0, then PG = 100 x
32
      (Mean OD(test) - Mean OD(tzero)/Mean OD(tzero)
33
34
35
```

Where: . 1 The average of optical density Mean OD (tzero) = measurements of SRB-derived colour just before exposure of cells to the test compound. 6 7 The average of optical density Mean OD (test) = 8 measurements of SRB-derived colour 9 after 48 hours with no exposure of 10 cells to the test compound. 11 12 The average of optical density Mean OD (ctrl) = 13 measurements of SRB-derived colour 14 after 48 hours with no exposure of 15 cells to the test compound. 16 17 It is clear from the results given in Tables 1 and 2 18 that uscharin has an inhibitory effect on the growth of 19 a wide variety of cancer cell lines in vitro. 20 21 EXAMPLE 5 22 23 IN VITRO SCREENING OF USCHARIDIN 24 25 Uscharidin was also subjected to in vitro cell 26 screening in the manner described in Example 4. 27 Results are given in Table 3 and Figure 3, and these 28 show that Uscharidin also exerts an inhibitory effect 29 on a variety of cancer cell lines in vitro. 30 31

EXAMPLE 6 1 IN VITRO SCREENING OF CALOTOXIN 3 4 5 Calotoxin was also subjected to in vitro cell screening 6 in the manner described in Example 4. Results are given in Table 4 and Figure 4, which show that 7 calotoxin also exerts an inhibitory effect on a variety. 8 of cancer cell lines in vitro. 10 11 EXAMPLE 7 12 13 IN VITRO EXPERIEMENT WITH USCHARIN IN NUDE MICE 14 15 The SCCI cells (human tumour cell line) where grown (1 16 x 10<sup>5</sup>/ml seeding density) in 25 ml RPMI 1640 (10% foetal 17 calf serum, 5% glutamine) in 75 cm2 tissue culture 18 The cells were harvested at log growth phase flasks. 19 (5 days approximately) and washed once in saline before 20 injection into the mice. 21 22 The "nude" mice (BALB/c nude) are reared and contained 23 within a sealed isolator. The mice were injected with 24 1 x 107 cells subcut on the back, right hand side near 25 the shoulder blades. After 7 days the mice were split 26 randomly into the study groups (10-15 animals per 27 group). Each was then treated with a different regime, 28 the variable being time between injections and dose of 29 drug at each injection, control groups were also 30 included in the overall plan of the experiement. 31 32 During the trial a daily check was made on the animals 33 and any animal removed if the tumour size became too 34 large (>5-7% total body weight) or if the animal is 35 showing signs of distress. Additional to this the

independent observer and the result recorded. Once an animal is removed from the study the tumour size, volume and weight was determined and the tumour stored for further cytological study. The reason for the animals removal from the study was also recorded, if this was not due to tumour size. The results are shown in the following tables.

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Using nude mice injected with 10' SCC-1 cells injected on day 0 and drug treatment started on day 9.

GROUP NO. 1 0.1 mg CGE-1/ Animal/ 5 days

			TUI	MOUR		
MOUSE	DAY REMOVED	VOL.	WEIGHT	RATE (mg/D)	NECROTIC (%)	REASON
A	27	4356.4	1.7492	64.8	22.41	1
В	55		NONE	_	·	5
С	30	4141.3	2.5658	85.5	45.28	1
D	30	299.8	1.8196	60.7	52.24	1
E	37	2752.8	1.5783	42.7	33.37	1
F	55	_	NONE	-	_	5
G	55	-	NONE		••	5
Н	55		NONE	-	-	5
I	33	3414.9	1.8805	57.0	28.69	1
J	55	-	NONE	-	-	5
K	37	828.9	0.6773	18.3	8.19	2
L	27	2223.8	1.6854	62.4	48.92	1
M	27	1556.2	0.7728	28.6	5.45	1
N	27	3457.9	1.9394	71.8	52.94	1
0	55	-	NONE	_		5
MEAN		2559.11	1.6298	54.64	33.05	
S.D.		1437.34	0.5844	21.20	18.29	

GROUP NO. 2

## 0.1 mg CGE-1/ Animal/ 10 days

			TU	MOUR		
MOUSE	DAY REMOVED	VOL. (mm³)	WEIGHT	RATE (mg/D)	NECROTIC (%)	REASON
А	27	2993.1	2.0570	76.2	49.92	1
В	55	· 	NONE	-	_	5
С	55	-	NONE	-	<b>.</b>	5
D	55	-	NONE	-	-	5
E	55	664.8	0.4333	7.9	17.91	5
F	55	3148.8	2.0378	37.1	16.96	5
G	55	134.4	0.1285	2.3	8.17	5
Н	55	-	NONE	-	<u>-</u> ·	5
I	55	-	NONE	-	_	5
J	.55	_	NONE	-	-	5
K	55	_	NONE	<b></b>	<b>-</b>	5
L	55	-	NONE	_	_	5
М	26	2025.9	1.3238	50.9	6.90	3
N	30	1548.8	1.2677	42.3	10.79	1
0	30	544.1	0.3827	12.8	25.29	4 .
MEAN		1579.99	1.0901	32.79	19.42	
S.D.		1201.27	0.7933	26.68	14.9	

GROUP NO. 3

0.5 mg CGE-1/ Animal/ 5 days

			T	JMOUR		
MOUSE	DAY	VOL. (mm³)	WEIGHT	RATE (mg/D)	NECROTIC (%)	REASON
A	55		NONE	_	-	5
В	55	219.6	0.2082	3.8	18.18	5
С	55	~	NONE	_	_	5
D	19	1494.7	1.1889	62.6	2.33	3
	19	203.2	0.0948	5.0	_	
E	19	<del>-</del>	NONE	_		3
F	23	3912.0	2.5341	110.2	13.13	1
G	28	4463.2	2.5717	91.8	23.42	1
Н	37		NONE	-	_	2
I	28	1666.5	1.0930	39.0	12.96	1
J	19	23.7	0.0038	0.2	=  =	3
K	33	1457.9	1.2546	38.0	19.22	1
L	23	1532.5	0.8926	30.8	12.49	1
M	29	2972.3	1.6348	56.4	17.79	1
N	37	537.9	0.4997	13.5	9.70	2
0	37	-	NONE	_	_	2
MEAN		1848.36	1.1976	45.12	14.36	
S.D.		1504.32	0.8738	36.61	6.18	

GROUP NO. 4

## 0.5 mg CGE-1/ Animal/ 10 days

			TU	MOUR		
MOUSE	DAY REMOVED	VOL.	WEIGHT	RATE (mg/D)	NECROTIC	REASON
A	28	1482.1	1.1211	40.0	28.48	1
В	27	3499.1	2.5087	92.9	32.54	1
С	42	1930.3	1.4088	33.5	13.58	1
D	42	2177.3	1.5067	35.9	17.14	1
Е	55	_	NONE	_	949	5
F	27	6882.3	3.1626	117.1	42.37	1
G	33	760.9	0.7467	22.6	50.31	1
Н	55	-	NONE	_	-	5
I	55	-	NONE			5
J	55	-	NONE	-	_	5
K	55	64:5	0.1127	2.0	17.78	5
L	29	-	NONE	_		2
М	55	-	NONE	-	-	5
N	23	4929.6	2.6126	113.6	37.52	1
0	55	_	NONE		-	5
MEAN		2715.76	1.6475	57.2	29.97	
S.D.		2272.64	1.0344	44.08	13.18	

GROUP NO. 5
CONTROL (0.1 ml Saline/ Animal/ 5 days

			TU	MOUR		
MOUSE	DAY REMOVED	VOL. (mm³)	WEIGHT	RATE (mg/D)	NECROTIC (%)	REASON
A	55	_	NONE	_	_	5
В	55	_	NONE	<b>-</b>	_	5
С	55	-	NONE	•		5
D	55	-	NONE		·	5
E	23	4570.9	2.4227	105.3	35.2	1
F	50	3138.3	1.9475	39.0	4.43	1
G	55	-	NONE	-	-	5
Н	55		NONE		_	5
I	3	-	NONE	-	_	3
J	23	5493.0	3.1602	137.4	59.07	1
K ;	28	2500.7	1.8958	67.7	6.68	1
L	28	3246.9	1.9716	70.4	31.86	1
M	55	_	NONE	-	<del>-</del>	5
N	28	4120.3	2.2965	82.0	46.07	1
О .	55	-	NONE	_		5
MEAN		3845.02	2.2707	83.63	30.55	
S.D.		1093.88	0.4797	34.01	21.59	

1	NOTES:-	
2	REASONS	3:
3	•	•
4	(1) Re	emoved due to tumour size.
5	(2) Re	emoved due to another illness.
6	(3) Fo	ound dead in cage.
7	(4) Re	emoved because the tumour was about to rupture.
ß	/51 R	emoved at end of the experiment.

TABLE 5
Table 5 gives a summary of the results.

	Tumour Growth (mg/day)	% Necrosis*	% Mortality at 40 days
Group 1 (0.1mg/5 days)	54.6 ± 21.1	33.1 ± 18.3	84
Group 2 (0.1mg/10 days)	32.8 ± 26.7	19.4 ± 14.9	55
Group 3 (0.5mg/5 days)	45.1 ± 36.6	14.4 ± 6.2	90
Group 4 (0.5mg/10 days)	57.2 ± 44.1	30.0 ± 13.2	62 -
Control	83.6 ± 34.0	30.6 ± 21.6	100

<sup>\*</sup> from histological examination Values are means  $\pm SD$ , n=15

From these results it can be seen that a reduction in percentage mortallity due to the cancer cells of up to 45% can be achieved by administration of the compound of the invention (Uscharin).

CT	A.	T	MS	
لليك	76.7	<u></u>		2

1

1. A composition comprising uscharin or analogues or salts thereof as active ingredient together with a pharmaceutically acceptable carrier or excipient.

6

7 2. The use of uscharin, analogues or salts thereof 8 for medical (including veterinary) purposes.

9

10 3. The use of uscharin as claimed in the preparation of a medicament.

12

13 4. A composition as claimed in Claim 1 or 2 wherein 14 the uscharin is suspended or dissolved in an 15 acceptable liquid carrier medium.

16

17 5. A composition as claimed in Claim 4 wherein the carrier medium is aqueous based.

19

20 6. A use as claimed in Claims 2 or 3 wherein 0.1-100 uscharin per kg body weight is used.

22

7. A method of treatment of a human or non-human animal body, said method comprising administering to said body a composition comprising uscharin.

26

27 8. A method as claimed in Claim 7 wherein a unit dose 28 of composition comprises between 20 and 500 mg 29 uscharin.

30

31

# 1/41

## Log10 Concentration

•	Time	·	Ме	an Op	tical De	ensitites	3
	Zero	Ctrl	-8.0	-7.0	-6.0	-5.0	-4.0
Panel/Cell Line	2610	Our					
Leukemia	0 279	0.993	0.912	0.166	0.134	0.134	0.124
CCRF-CEM		1.228	1.324	0.102	0.104	0.100	0.102
HL-60(TB)		0.825	0.904	0.152	0.085	0.104	0.111
K-562 MOLT-4		1.577	1.463	0.194	0.163	0.151	0.337
RPMI-8226		1.374					0.276
SR		1.450	1.279	0.138	0.127	0.094	0.150
Non-Small Cell Lung Cancer							0.000
A549/ATCC	0.381	1.657					0.098
EKVX	1.154	1.728	1.790	0.617	0.244	0.352	0.162
HOP-18				0.000		. 0 040	0.014
HOP-62		1.702	1.699	0.208	0.030	0.010	0.014
HOP-92		0.957	0.970	0.554	0.208	0.214	0.173
NCI-H226	<del>-</del>	1.325	1.36/	0.574	2 U. 198	0.220	0.093 0.250
NCI-H23		5 1.407					0.230
NCI-H322M		4 1.480	1.518	0.02	) 0.40 1 0.011	2 -U UU	2 0.018
NCI-H460	0.177	7 1.224	1.10	0.03	J 0.0 i	, -U.UU.	2 0.010
	0.47/	0 762	0.720	0 13	0 0 04	4 0.068	3 0.098
NCI-H522		6 0.763 6 1 495					2 0.015
LXFL 529	U.45t	6 1.485	1.400	0.00	. 0.0		
Small Cell Lung Cancer	0.44	0 1.308	0.710	0.20	4 0.10	0 0.15	8 0.116
DMS 114		6 1.331	1.34	2 -0.00	1-0.01	2 0.01	3 0.016
DMS 273	0.20	0 1.001					
Colon Cancer COLO 205	0 27	7 1.284	1.21	5 0.30	0.08	7 0.18	6 0.091
DLD-1		3 0.866	0.84	4 0.03	5 0.02	6 0.01	2 0.030
HCC-2998		6 0.817					4 0.010
HCT-116		5 1.376					1 0.069
HCT-15		8 1.790					7 0.060
HT29		8 1.271	1.34	2 0.22	21 0.05	0.04	6 0.038
KM12							0.007
KM20L2	0.26	34 1.047	1.05	4 0.15	52 0.01	2 0.00	0.007
SW-620	0.22	9 1.324	1.29	9 0.17	79 0.07	4 0.13	34 0.133
CNS Cancer		•	4.46		00.00		10 0 003
SF-268		96 1.240	1.10	9 0.3	38 0.04	19 U.U <sup>2</sup>	19 0.093
SF-295		03 1.521	1.53	6 U.40	J9 U.SU	)	71 0.078
SF-539		16 1.793	1./0	$\frac{12}{12}$ 0.30	)4 U.U	51 U.US	93 0.113 98 0.337
SNB-19		56 1.894					14 0.380
SNB-75		64 0.864	0.81	1 U.S	74 O.31	25 0.4 26 0.40	0.363
SNB-78		57 1.093	1.1	10 0.4	14 U.4.	20 0.40 16 0.01	0.018
U251		69 1.179	1.24	24 U.U	62 0.0	10 0.00	14 0.015
XF 498	0.40	69 0.713		10 U. I	UZ U.U	72 U.U	14,0.010
•		Fig. 1a					
	-	•					

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## Log10 Concentration

	Time		Me	an Opt	cal De	nsitites	
Panel/Cell Line	Zero	Ctrl	-8.0	-7.0	<b>-</b> 6.0	-5.0	-4.0
Melanoma	0.256	1 365	1 346	0.016	-0.001	0.017	0.016
LOX IMVI MALME-3M	0.230		<del>-</del>	0.235			
MACINE-21VI	0.044	1.200		<del>-</del> • • • • •			
M14	0.333	1.167		0.240			
M19-MEL	0.284	1.126		0.386			
SK-MEL-2	0.570	1.357	•	0.280			
SK-MEL-28	0.254	0.562		0.278			
SK-MEL-5	0.485	1.905	1.896	0.249	0.200	0.179	0.134
•			0.447	0.070	0.400	0.465	0 0 2 4 4
UACC-257		2.040					0.344
UACC-62	0.516	1.714	1.649	0.465	0.103	U. 103	0.055
Ovarian Cancer	0.444	4 277	4 422	0.510	0 257	n 302	0.320
IGROV1	_	1.377		0.310			
OVCAR-3		1.189 1.051					0.004
OVCAR-4		0.848					0.012
OVCAR-5		1.784					0.206
OVCAR-8 SK-OV-3		1.165					0.122
Renal Cancer	0.400	1	,,,,,,,				
786-0	0.274	1.093	1.062	0.027	0.011	0.008	0.027
A498		3 1.360	1.348	0.872	0.718	0.544	0.341
ACHN		2 1.349	1.204	0.130	0.024	0.020	0.069
CAKI-1							
RXF-393	0.856	3 1.266	1.203	0.613	0.391	0.499	0.668
RXF-631							
SN12C	0.239	1.533					0.040
TK-10		1.057					0.088
UO-31	0.789	9 1.347	1.424	1 0.715	0.462	2 U.46t	6 0.607

Log 10 Concentration

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			)						
•			Perce	ent Gro	wth				
Panel/Cell Line	-8.0	-7.0	-6.0	-6.0 -5.0 -4.	4.0	G150	TGI	LC50	
Leukemia	Ç	7	C	Ç	, u	1 00E-08	4 86F-08	6.73E-07	
CCRF-CEM	D X	1.5-	7C-	70-	9 1	1.00 L.00 L.00 L.00 L.00 L.00 L.00 L.00	20 Had. 4	7 62E_08	
HL-60(TB)	<del></del>	-71	-71	-72	-/1	2.16E-U8	4.UDE-U0	00-150.7	
K-562	111	4	-30	-13	-7	3.74E-08	1.35E-07	>1.00E-04	
	06	-60	-67	-69	-31	1.83E-08	3.95E-08		
	07	72	-48	-42	49	2.45E-08	6.34E-08	>1.00E-04	
SR SR	8 8	-60	\$ <b>\$</b>	-73	-57			8.48E-08	
Non-Small Cell Lung Cancel	ē				•			L	
) U	95	-65	-80	-71	-74		3.92E-08	.04E	
EKVX	111	-47	-79	-70	-86	2.43E-08	5.06E-08	1.28E-0/	
HOP-18								1	
HOP-62	100	-76	96-	-98 -08	-98	1.92E-08	3.70E-08	12F	
HOP-92	104	-13	-58	99-	-73	2.89E-08	7.75E-08	6.75E-U/	
NCI-H226	110	-38	-78	9/-	<u>6</u> -	2.56E-08	5.57E-08	2.01E-07	
NOT HON	77	-83 83	-84	-70	-52	1.47E-08	3.02E-08	6.21E-08	
NCI-H322M	104	ဖ	-18	-38	-52	3.57E-08	1.79E-07	7.60E-05	
	9	-83	-63	-100	06-	1.80E-08	3.43E-08	6.52E-08	
	α	73	6	-86	08-	1.73E-08	3.53E-08	7.23E-08	
NCI-H322	3 5	2 æ	- 0 - 0 -	70-	-97	1.86E-08	3.41E-08	6.27E-08	
Small Cell Lung Cancer	•		i Y						
	31	•	-77	-64	-74	<1.00E-08	3.74E-08	3.12E-0/	
DMS 273	101	-100		-95	-94	1.79E-08	3.18E-08	5.64E-08	
Colon Cancer									
COLO 205	93	7	<del>-</del> 00	-32	-67	2.98E-08	1.08E-07		
1-010	97	-77	-83	-92	-80	1.86E-08	3.59E-08	6.96E-08	
HCC-2998	118	ဖ	-63	66-	-97	4.03E-08	1.15E-07	3.68E-07	
HCT-116	06	9-	-93	-87	-71	1.85E-08	3.98E-08	8.55E-08	
HCT-15	106	-77	-77	88-	<del>-</del> 64	2.03E-08	3.81E-08	7.16E-08	

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LC50	3.70E-07	1.38E-07	2.06E-07 3.39E-07 8.11E-08	4.43E-05 >1.00E-04	>1.00E-04 7.11E-08 8.04E-08	5.91E-08 8.26E-08 2.31E-07	9.88E-08 3.56E-05 1.36E-07	6.40E-05 3.73E-07	>1.00E-04 9.05E-08 6.05E-08
TGI L	8.05E-08	5.05E-08 1 6.57E-08		_	7.50E-08 3.78E-08 3.99E-08	3.25E-08 4.08E-08 6.03E-08	1.42E-07 4.50E-08 1.80E-07	1.72E-07 8.04E-08	1.39E-07 4.53E-08 3.15E-08
	3.03E-08	2.27E-08 2.51E-08	1.92E-08 2.30E-08 1.83E-08	4.03E-08 2.53E-08	2.86E-08 2.00E-08 1.98E-08	1.78E-08 2.01E-08 2.44E-08	3.69E-08 2.05E-08 4.03E-08	2.16E-08 3.86E-08 2.67E-08	3.63E-08 2.27E-08 1.64E-08
wth -4.0	-84	-97	-8- -89 7	9 6 23 23	85. 89. 79.	94 98 99 99	-49 -64	-72 -53 -82	-28 -61 -99
nt Gro	<del>6</del> 4	-97	-90 -76	-30 -30	-27 -27 -96 -97	9. 9. 9. 9.	8 8 8 8 8 8	-63 -37 -68	-32 -95 -99
Percent Growth -6.0 -4.0	-80	င်္ လို လို			-24 -24 -24 -24 -25 -26 -26 -26 -26 -26 -26 -26 -26 -26 -26	0	-67 -84 -22	-34 -80 -80	-42 -51
-7.0 -					-15 -77 -66		8 -5.7	-49	7 -57 -88
بة 0.6	•				405 105 105 99		96 105 115	99 106 95	105 109 88
ani I ila Mina	Colon Cancer	KM12 KM20L2	SW-520 CNS Cancer SF-268 SF-295	- M	က္ထ	XF 498 Melanoma LOX IMVI MALME-3M	M19-MEL SK-MEL-2	SK-MEL-5 UACC-257	UACC-02 Ovarian Cancer IGROV1 OVCAR-3 OVCAR-4

6.11E-08 3.58E-07 >1.00E-04

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1 <u>G</u>	3.42E-08 7.60E-08 9.72E-08	3.28E-08 3.38E-06 3.57E-08	5.61E-08	3.34E-08 1.10E-07 8.40E-08
GI50	1.86E-08 2.79E-08 2.75E-08	1.77E-08 5.66E-08 1.68E-08	2.02E-08	1.83E-08 3.38E-08 3.30E-08
owth -4.0	-97 -67	90 45 83	-22	-83 -86 -23
Percent Growth 3.0 -5.0 -4.0	9 9 4 8 4 8	-97 -12 -95	-42	-92 -94 -41
Y	-95 -64 -64	-96 -94	-54	-94 -41
-7.0	-14	-90 -34 -68	-28	9. 6.
<b>8</b> .0	101 104 90	9 9 9 9 9	84	100 102 114
Panel/Cell Line	Ovarian Cancer OVCAR-5 OVCAR-8 SK-OV-3	Renal Cancer 786-0 A498 ACHN	CAKI-1 RXF-393	KAF-631 SN12C TK-10 UO-31

6.09E-06 >1.00E-04 7.56E-08

6.31E-08 3.22E-07

**LC50** 

Fig. 1e

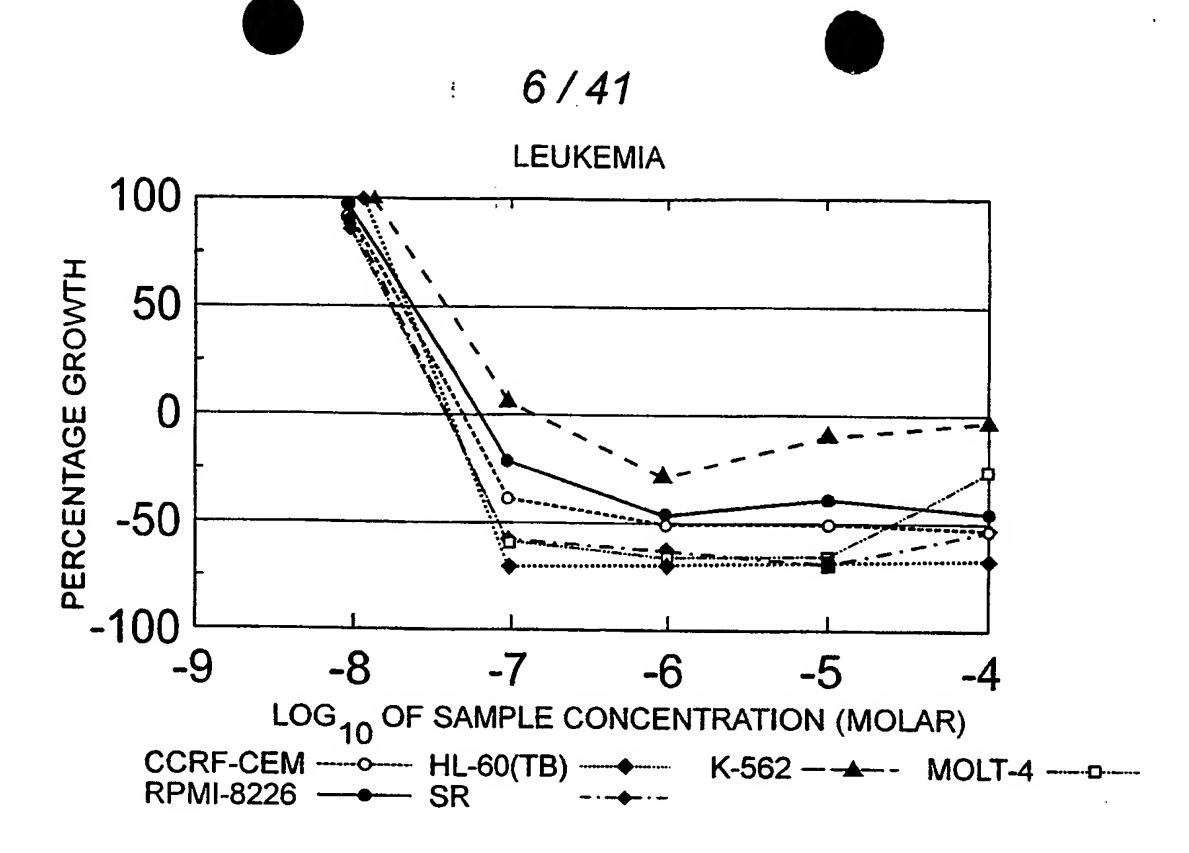
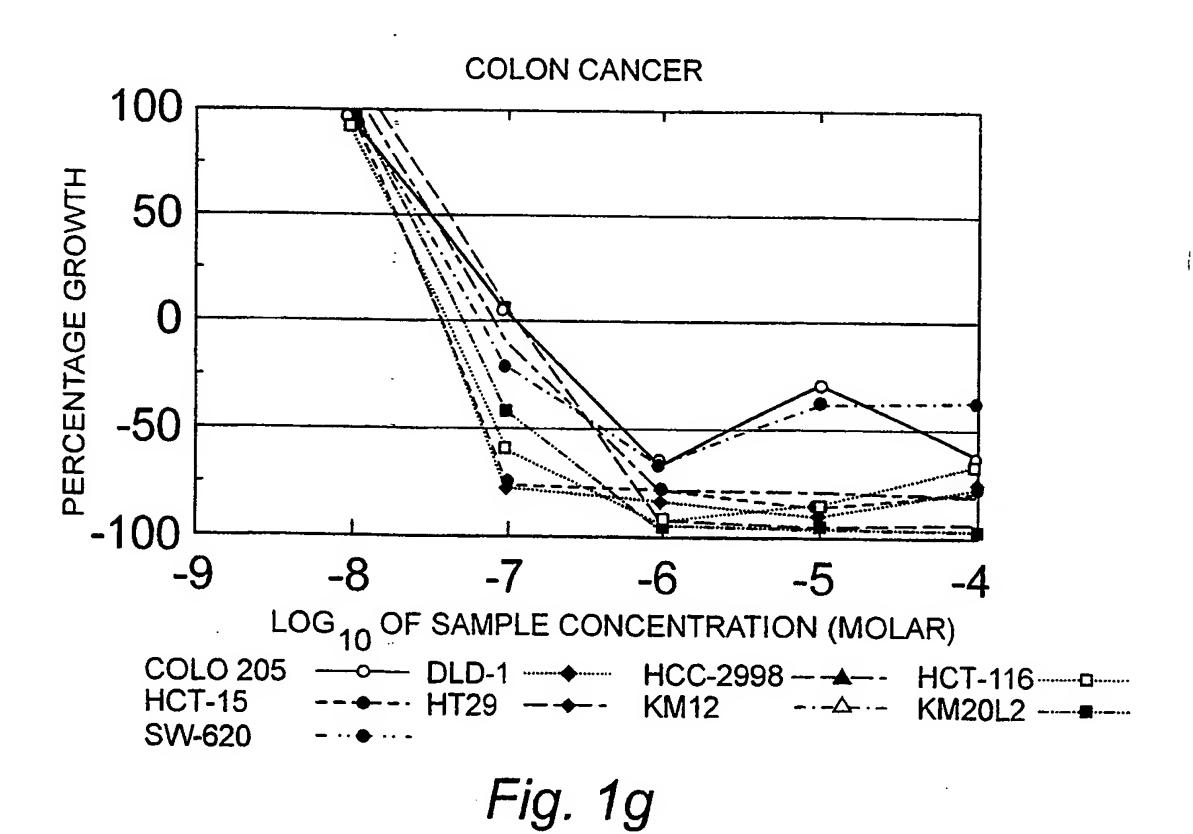
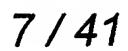


Fig. 1f



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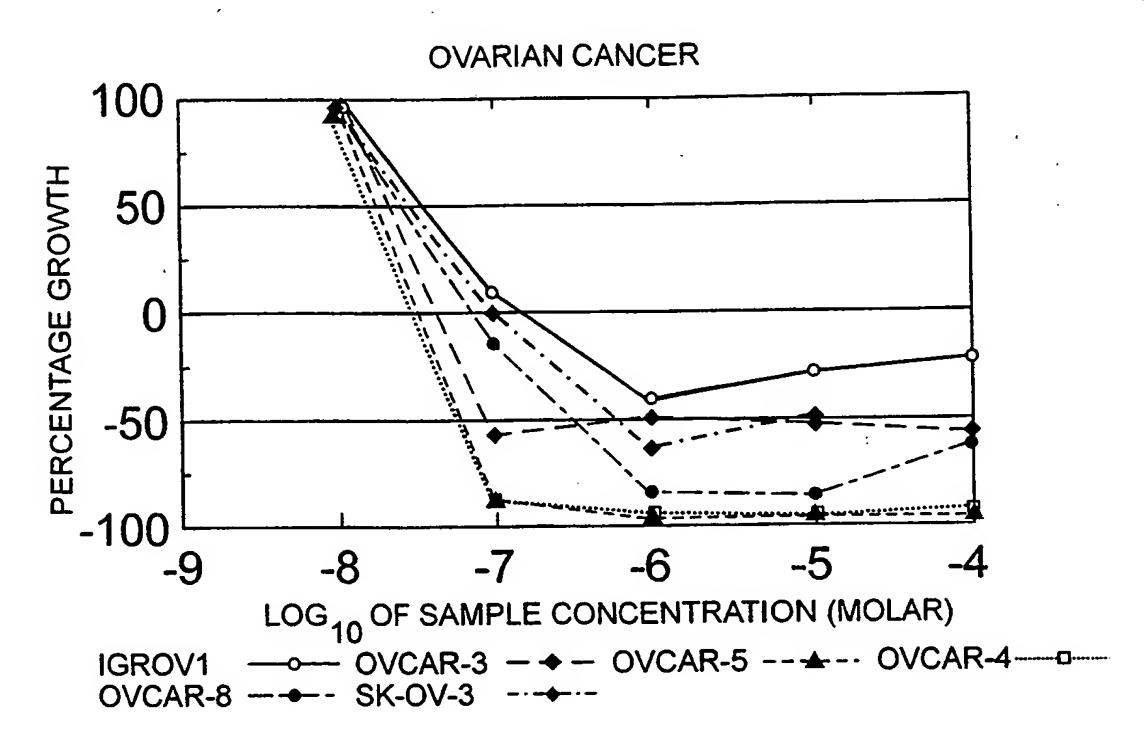


Fig. 1h

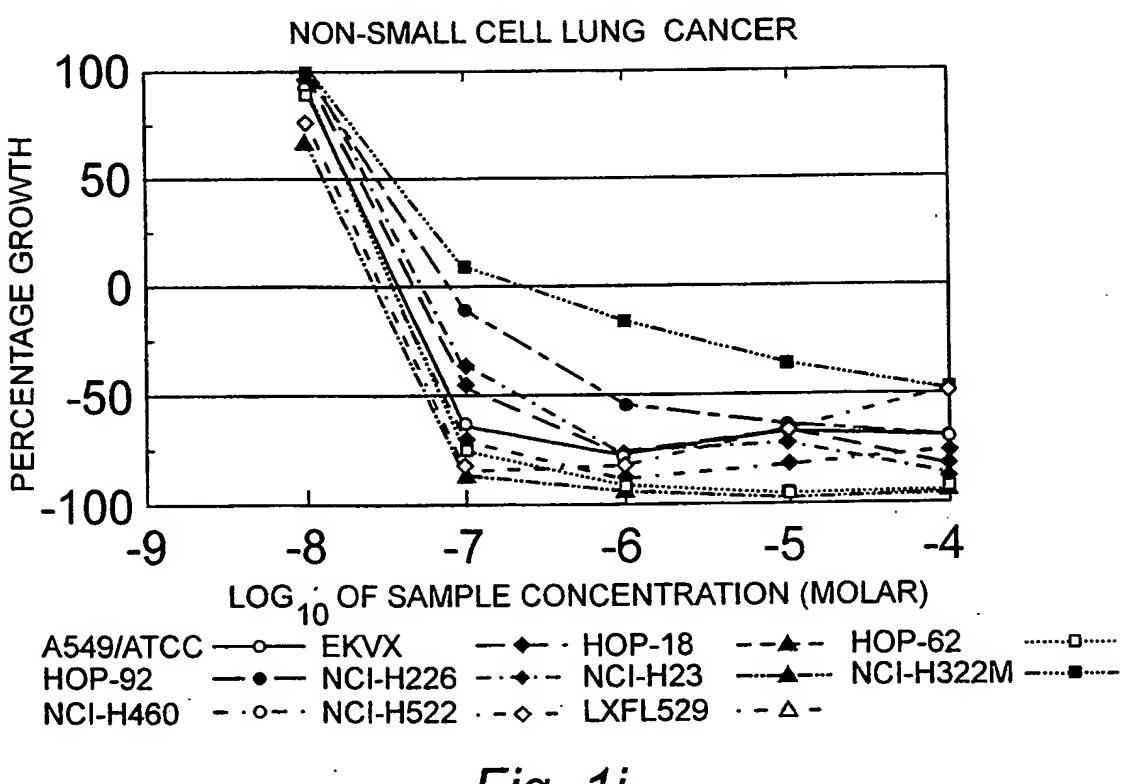


Fig. 1i

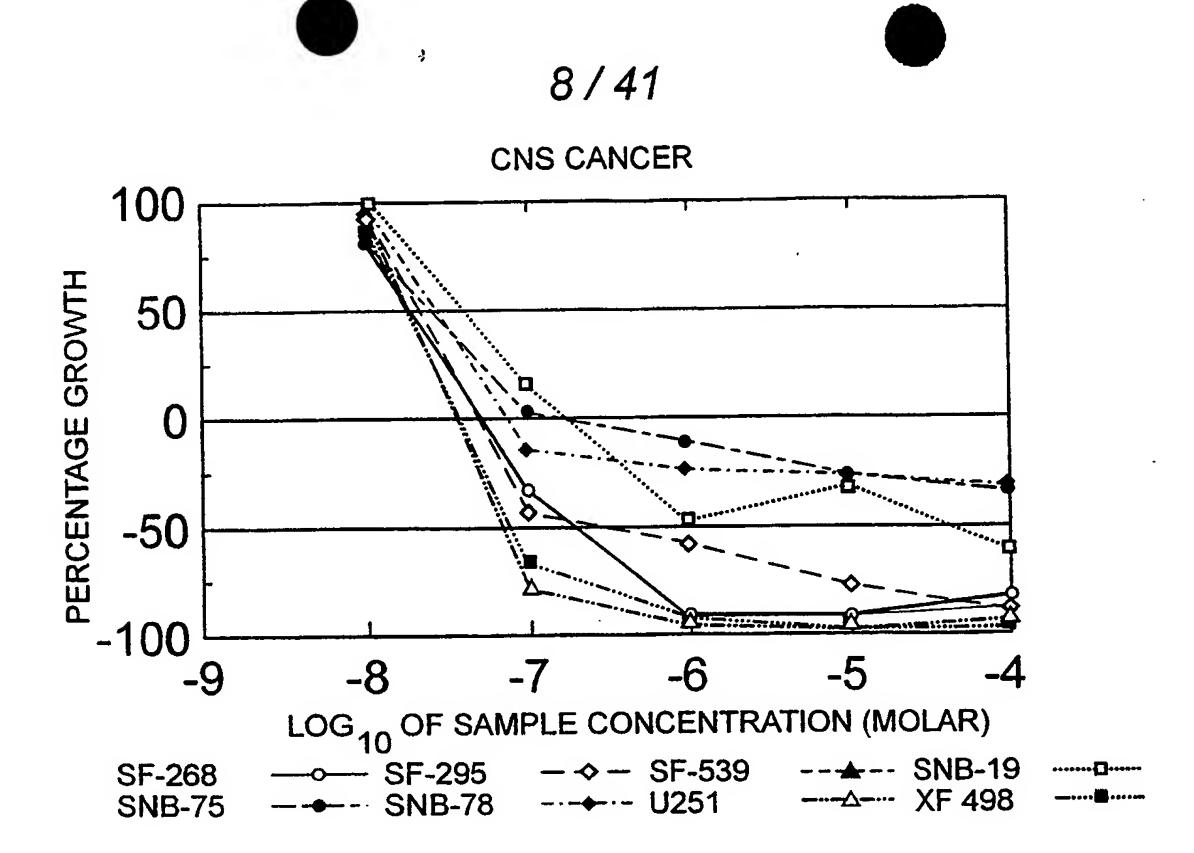


Fig. 1j

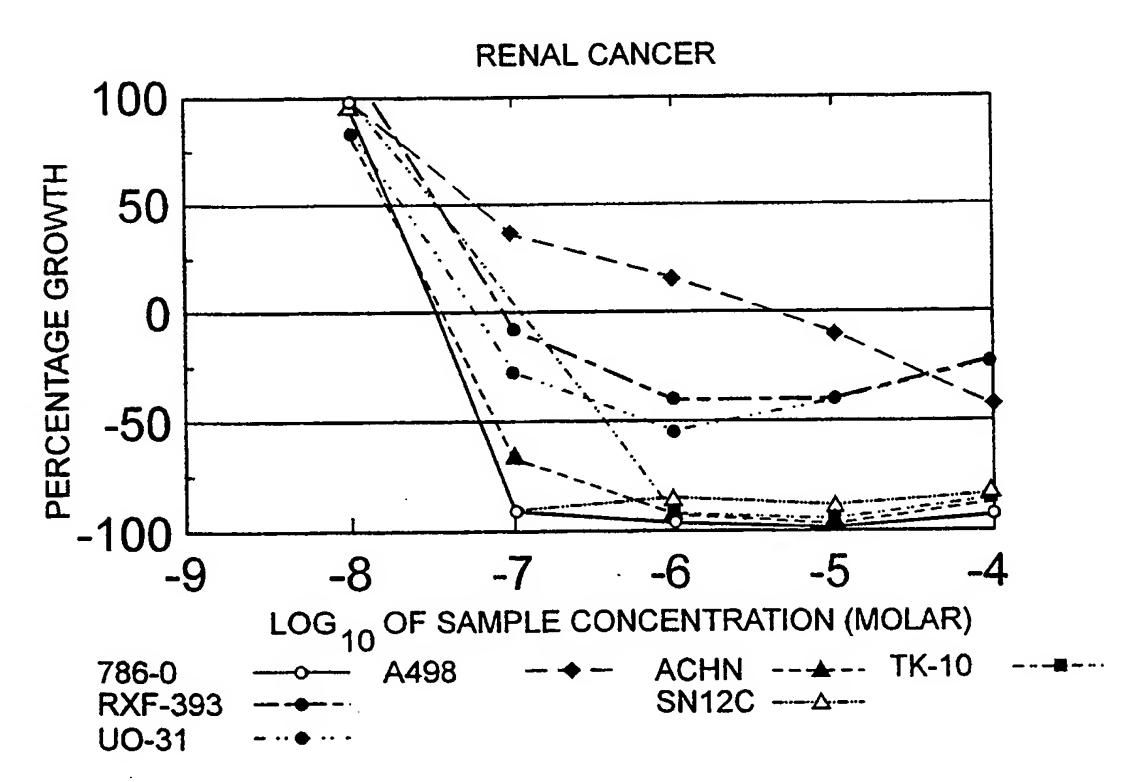


Fig. 1k

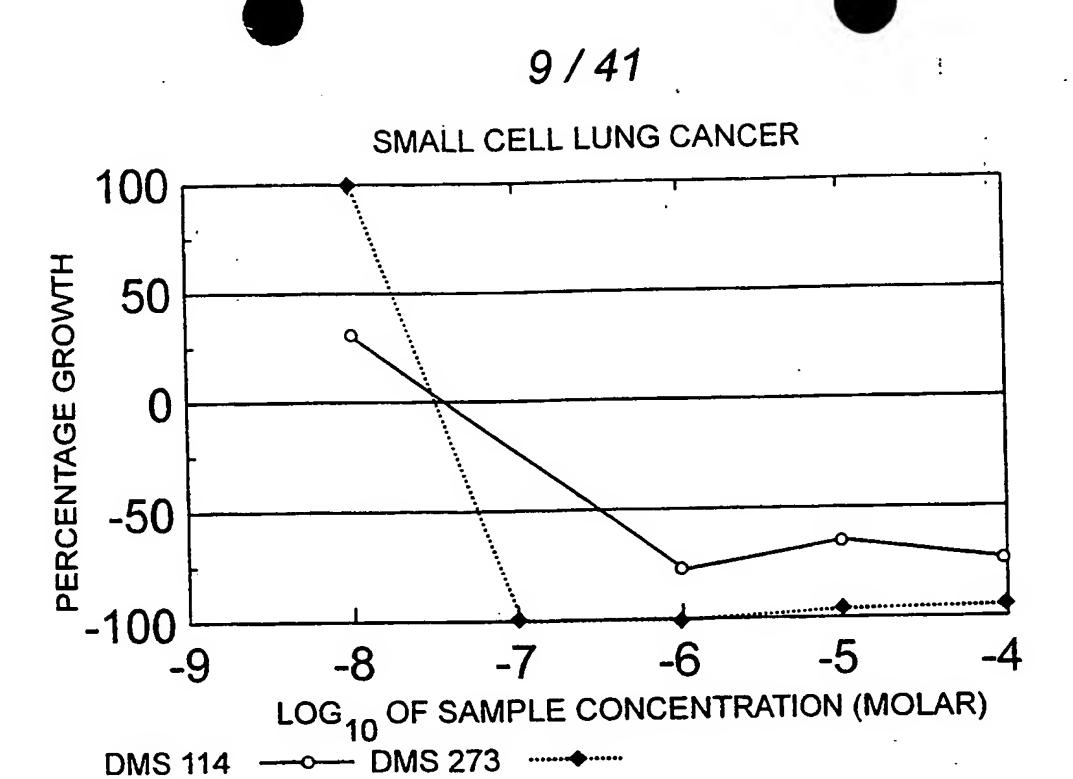


Fig. 11

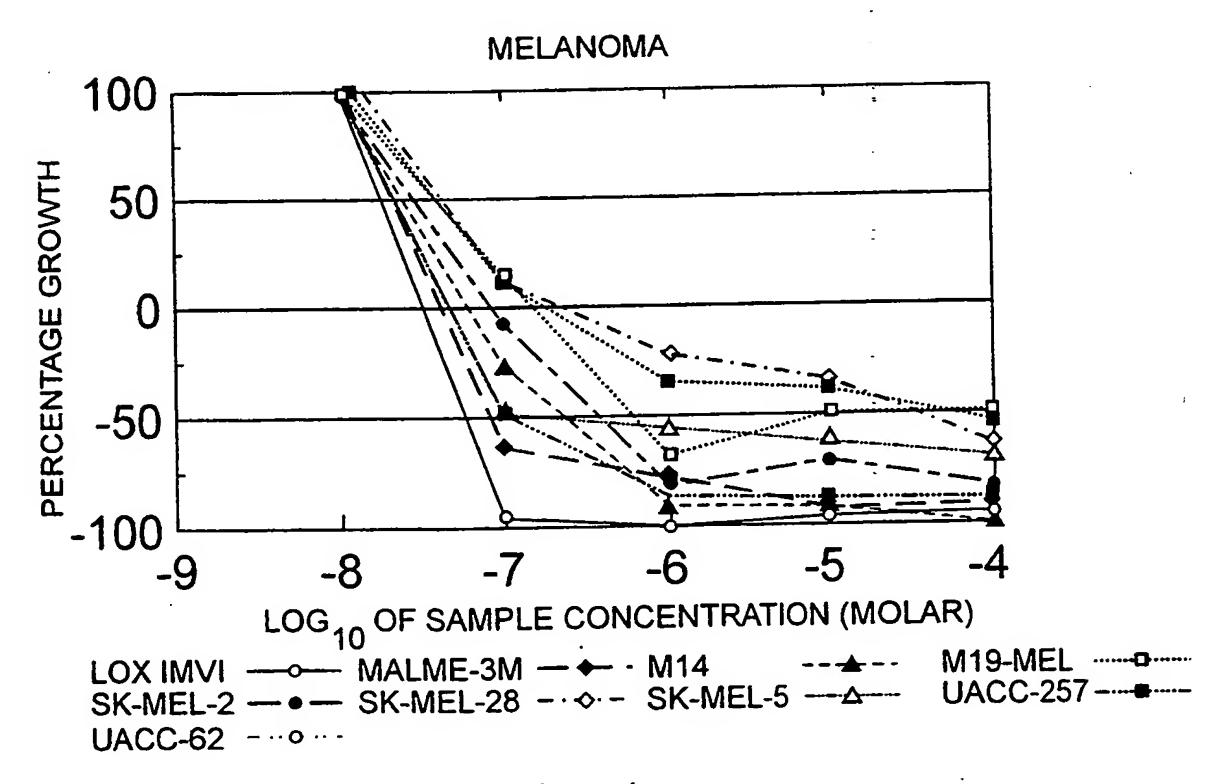


Fig. 1m

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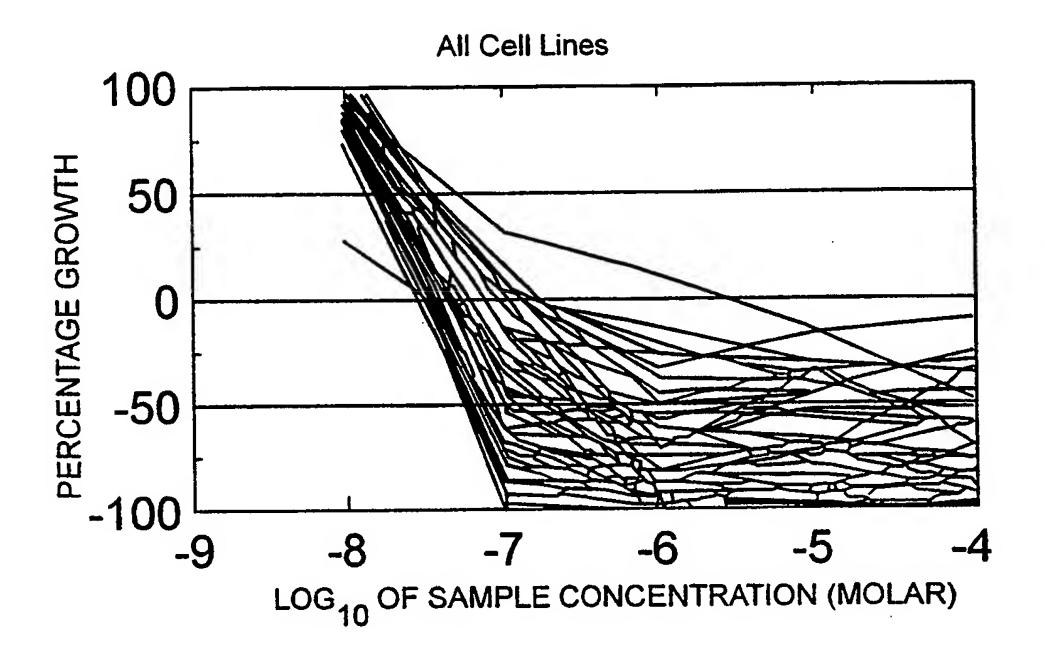


Fig.:1n

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National Cancer Institute Developmental Therapeutics Program	NSC: D-654033-0/I		Units: Molar SSP	L:0CXW	Exp. ID: 9207SC8	
Mean Graphs	Report Date: September 8, 1992	ber 8,	Test Date: July 20,	, 1992		
Panel/Cell Line	Log <sub>19</sub> GIS0 G1S0	20	Log <sub>19</sub> TGI	rgi	Logio LC50	LC50
Leukemia						
CCRF-CEM	-7.70		-7.31		-6.17	
HL-60(TB)	-7.67		-7.39		-7.12	
K-562	-7.43		-6.87		> 4.00	
MOLT-4	-7.74		-7.40			
RPMI-8226	-7.61		-7.20		> -4.00	
SR	-7.76		-7.42		-7.07	
Non-small Cell Lung Cancer						
A549/ATCC	-7.72		-7.41		-7.09	
EKVX	-7.61		-7.30		-6.89	
HOP-18					•	
HOP-62	-7.72		-7.43		-7.15	
HOP-92	-7.54		-7.11	- 55	-6.17	
NCI-H226	-7.59		-7.25		-6.70	
Fig. 10	+3 +2 +1 0	-1 -2 -3	+3 +2 +1	0 -1 -2 -	3 +3 +2 +1 (	0 -1 -2 -

	2	/	4	1
•		,	•	

NCI-H23	-7.83	-7.52	-7.21
NCI-H322M	-7.45	-6.75	4.12
NCI-H460	-7.74	-7.46	-7.19
NCI-H522	-7.76	-7.45	-7.14
LXFL 529	-7.73	-7.47	-7.20
Small Cell Lung Cancer			
DMS 114	< -8.00	-7.43	-6.51
DMS 273	-7.75	-7.50	-7.25
Colon Cancer			
COLO 205	-7.53	-6.97	
DLD-1	-7.73	-7.44	-7.16
HCC-2998	-7.39	-6.94	-6.43
HCT-116	-7.73	-7.40	-7.07
HCT-15	-7.69	-7.42	-7.15
HT29	-7.52	-7.09	-6.43
KM12			
KM20L2	-7.64	-7.30	-6.86
SW-620	-7.60	-7.18	
CNS Cancer			
Fig. 1p	+3 +2 +1 0 -1 -2	-3 +2 +1 0 -1 -2 -	3 +2 +1 0 -1 -2

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SF-268	-7.72	-7.28		-6.69
SF-295	-7.64	-7.29		-6.47
SF-539	-7.74	-7.41		-7.09
SNB-19	-7.39	-6.74		4.35
SNB-75	-7.60	-6.80		> 4.00
SNB-78	-7.54	-7.12		> 4.00
U251	-7.70	-7.42		-7.15
XF 498	-7.70	-7.40		-7.09
Melanoma				
LOX IMVI	-7.75	-7.49		-7.23
MALME-3M	-7.70	-7.39		-7.08
M14	-7.61	-7.22		-6.64
M19-MEL	-7.43	-6.85		
SK-MEL-2	-7.69	-7.35	- 50	-7.01
SK-MEL-28	-7.39	-6.74		4.45
SK-MEL-5	-7.67	-7.33		-6.87
UACC-257	-7.41	-6.76		4.19
UACC-62	-7.57	-7.09		-6.43
Ovarian Cancer	-	-		
Fig. 1q	+3 +2 +1 0	-1 -2 -3 +	+3 +2 +1 0 -1 -2	3 +3 +2 +1 0 -1 -2

<del> </del>	<del>                                     </del>	<del>T</del>	1	T				<del> </del>	<del></del>	14	/4	11			<u>.</u>		<del></del>		aj (
	- 50																		- 4
> 4.00	1 9	-7.22	-7.20	-6.49			-7.22	> 4.00	=				-7.21	-6.45	> 4.00	-6.30	0.95	3.25	
								·											- T
-6.86	-7.34	-7.50	-7.47	-7.12	-7.01		-7.48	-5.47	-7.45		-7.25		-7.48	-6.96	-7.08	-7.21	0.31	2.05	+3 +2 +1 (
																·			-1 -2 -3
-7.44	-7.64	-7.79	-7.73	-7.55	-7.56		-7.75	-7.25	-7.77		-7.69		-7.74	-7.47	-7.48	-7.63	0.37	0.75	+3 +2 +1 0
IGROVI	OVCAR-3	OVCAR-4	OVCAR-5	OVCAR-8	SK-OV-3	Renal Cancer	786-0	A498	ACHN	CAK1-1	RXF-393	RXF-631	SN12C	TK-10	UO-31	MG MID	Delta	Range	Fig. 1r

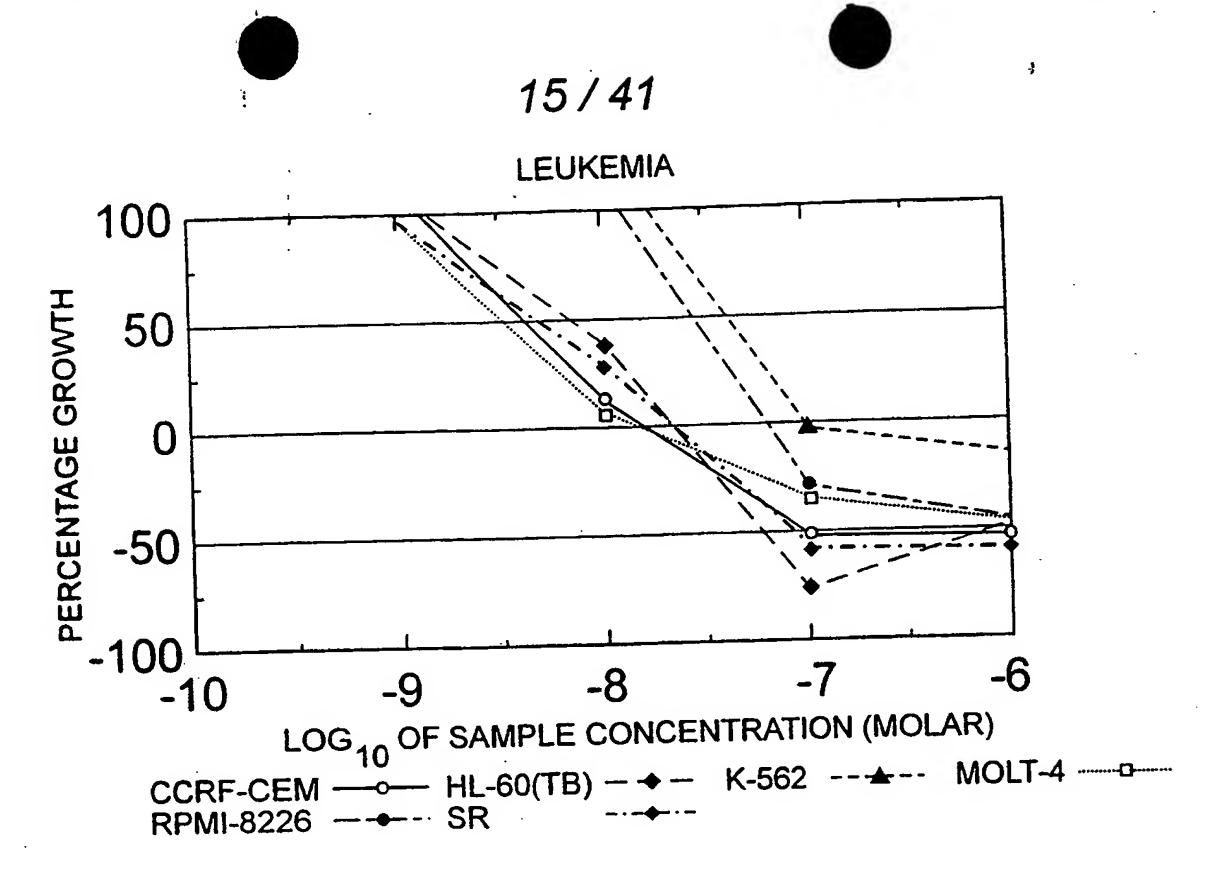


Fig. 2a

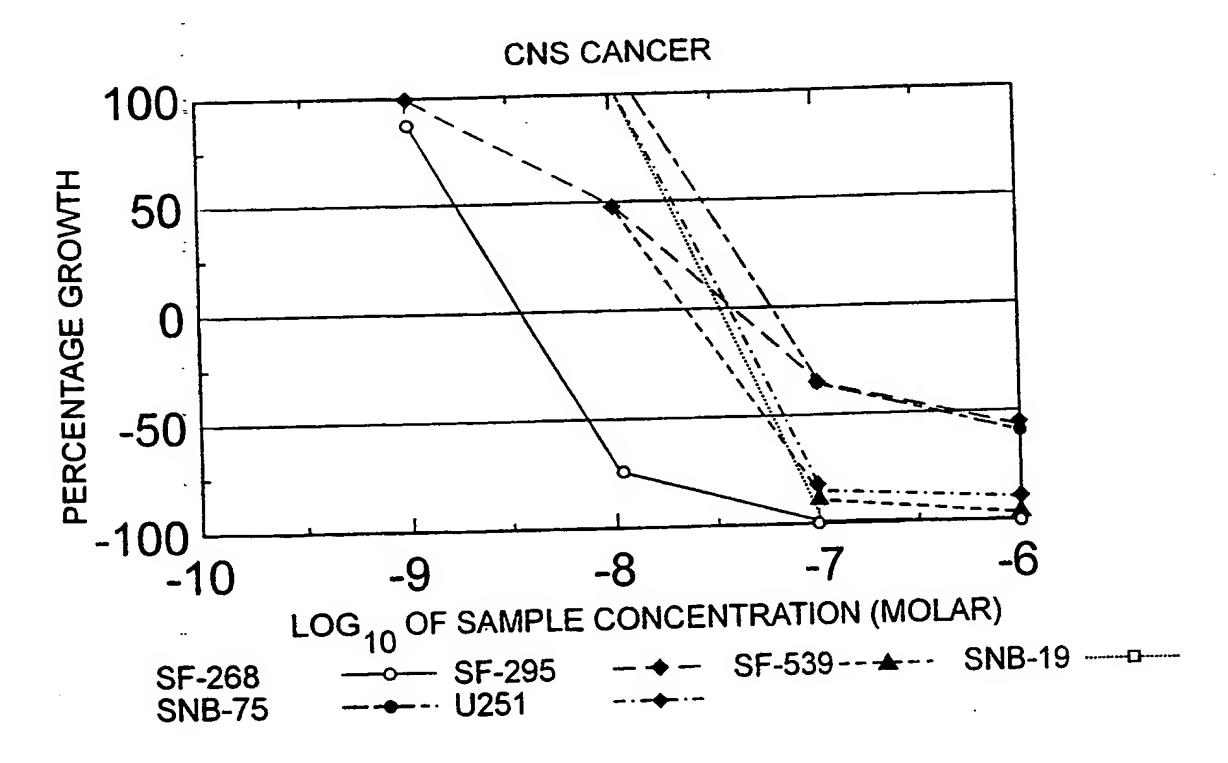


Fig. 2b

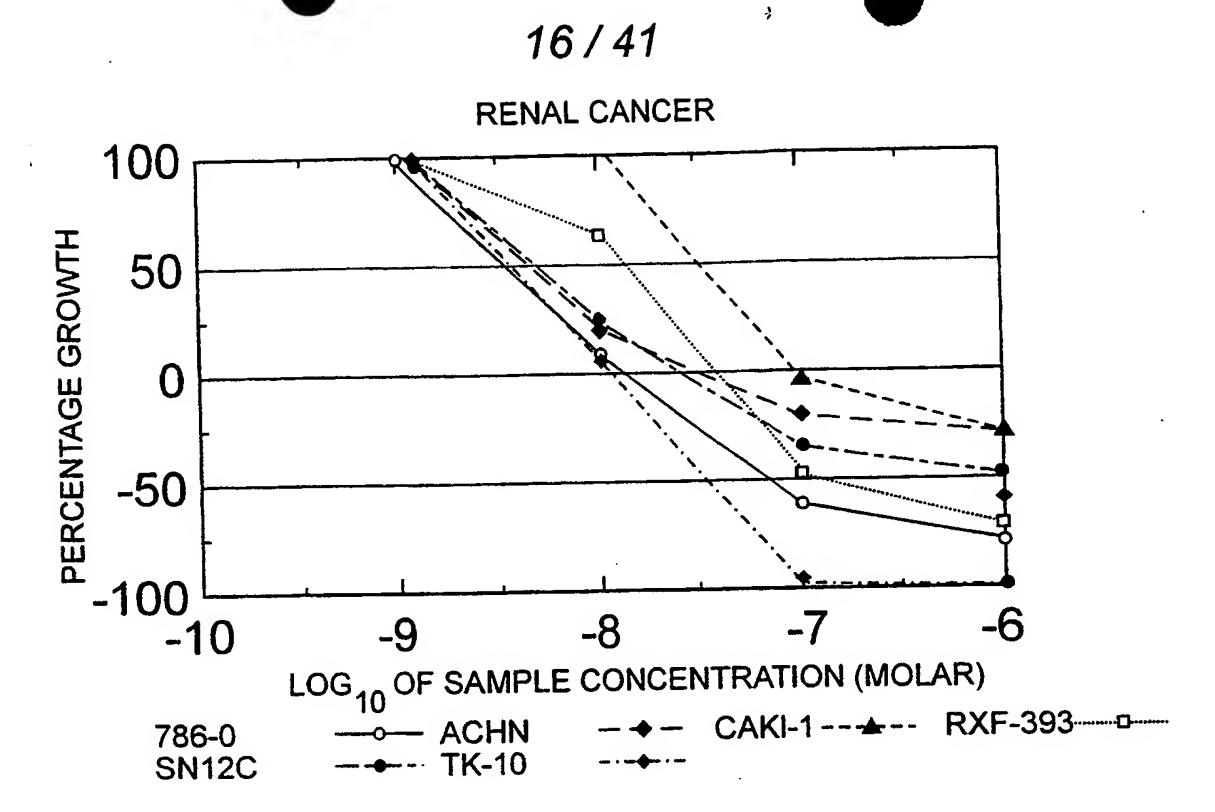


Fig. 2c

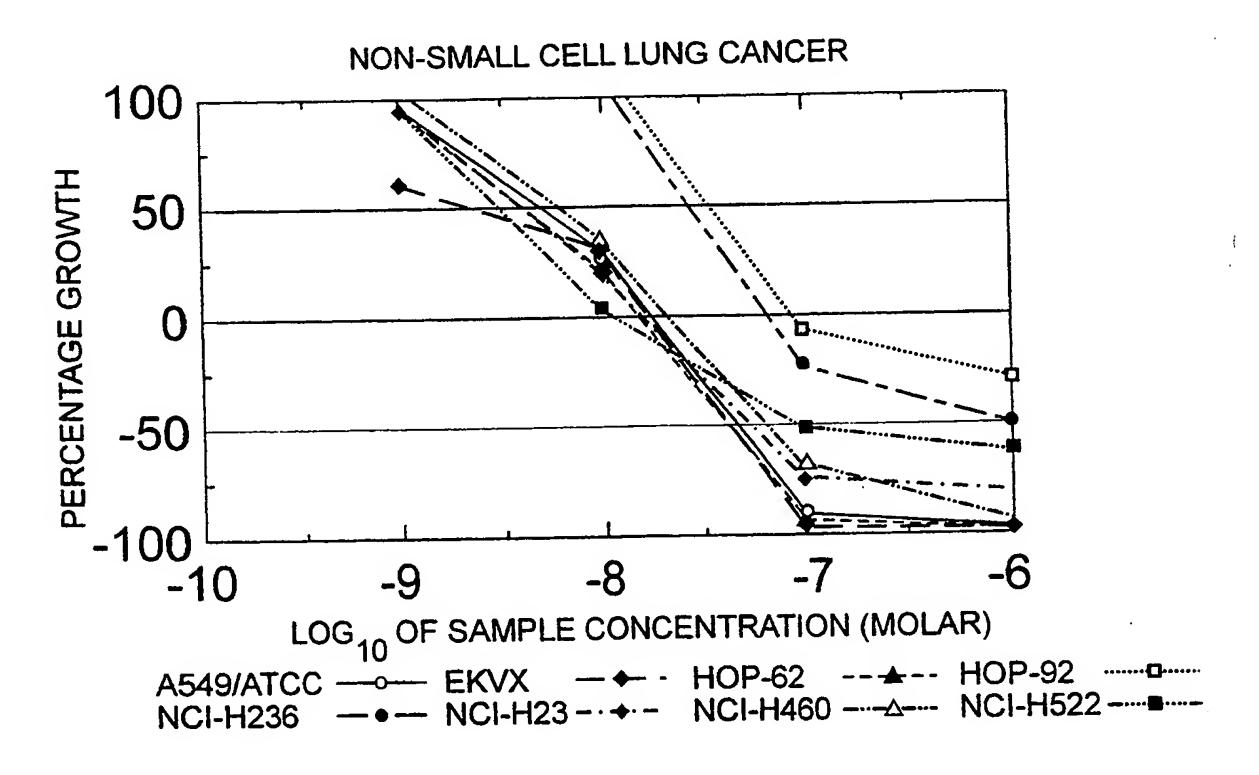


Fig. 2d

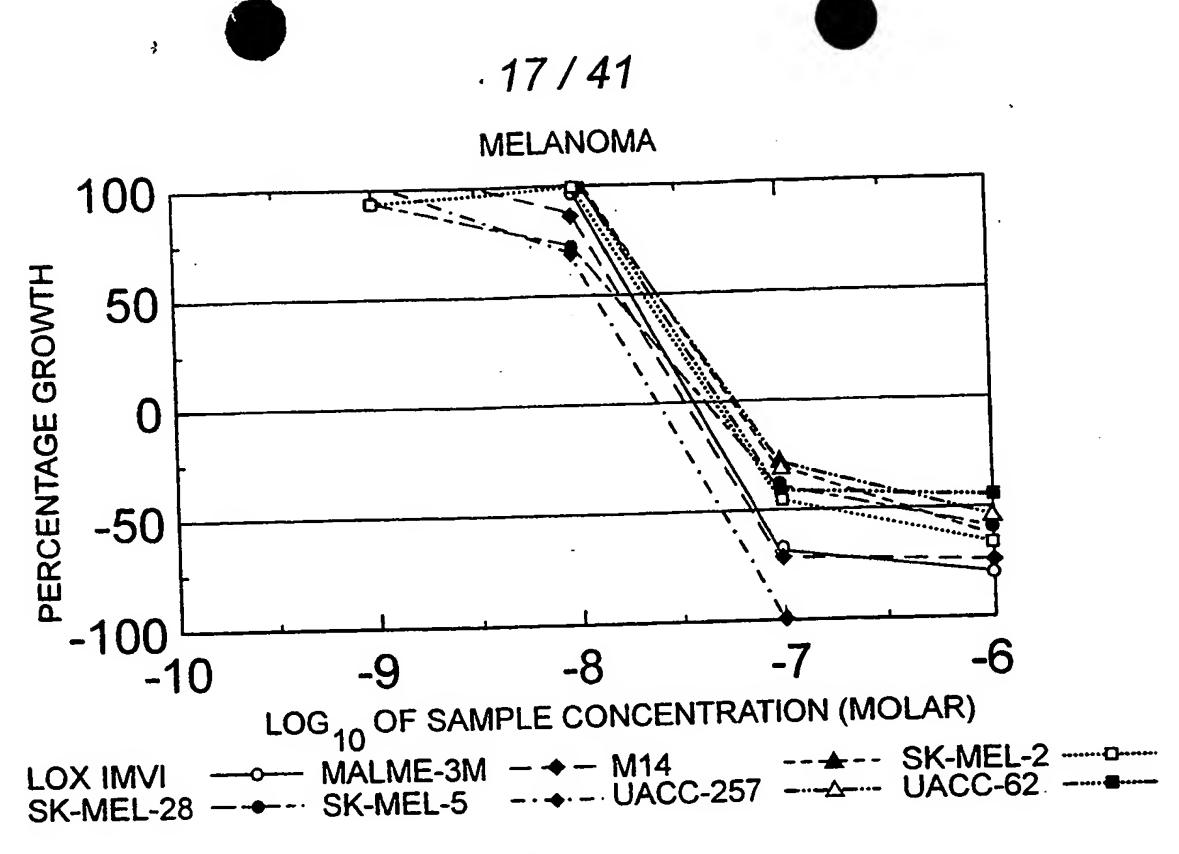


Fig. 2e

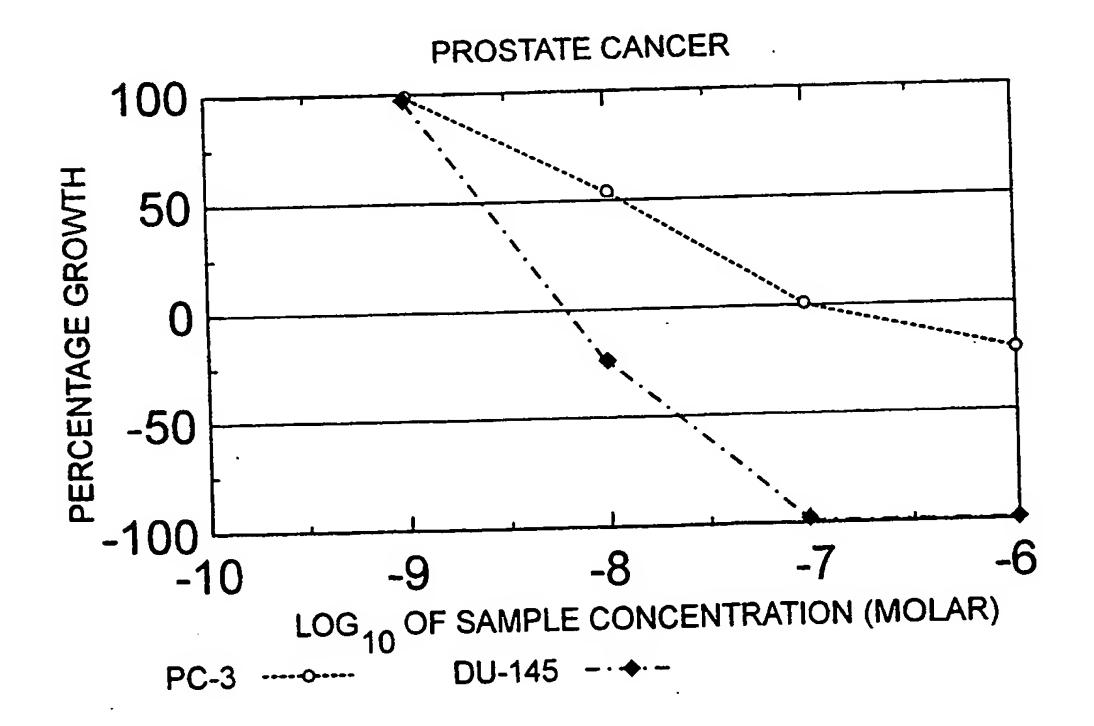


Fig. 2f

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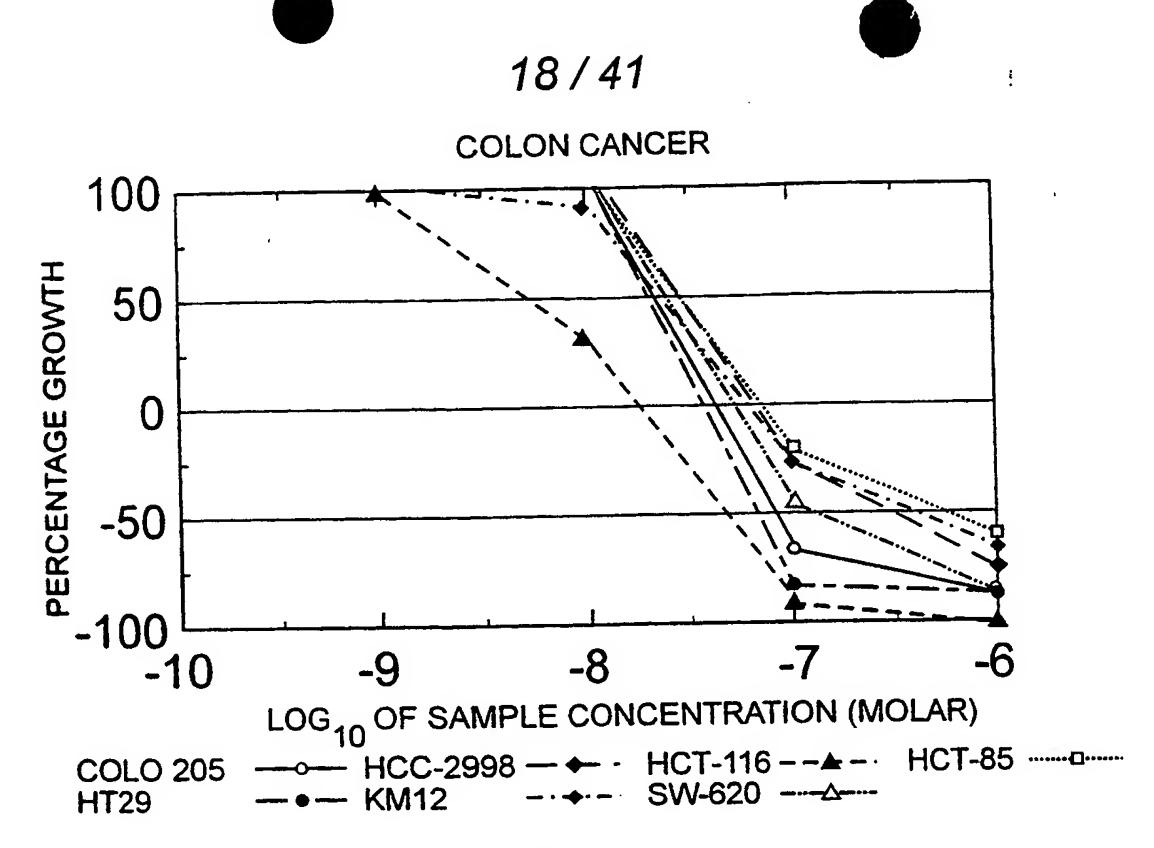


Fig. 2g

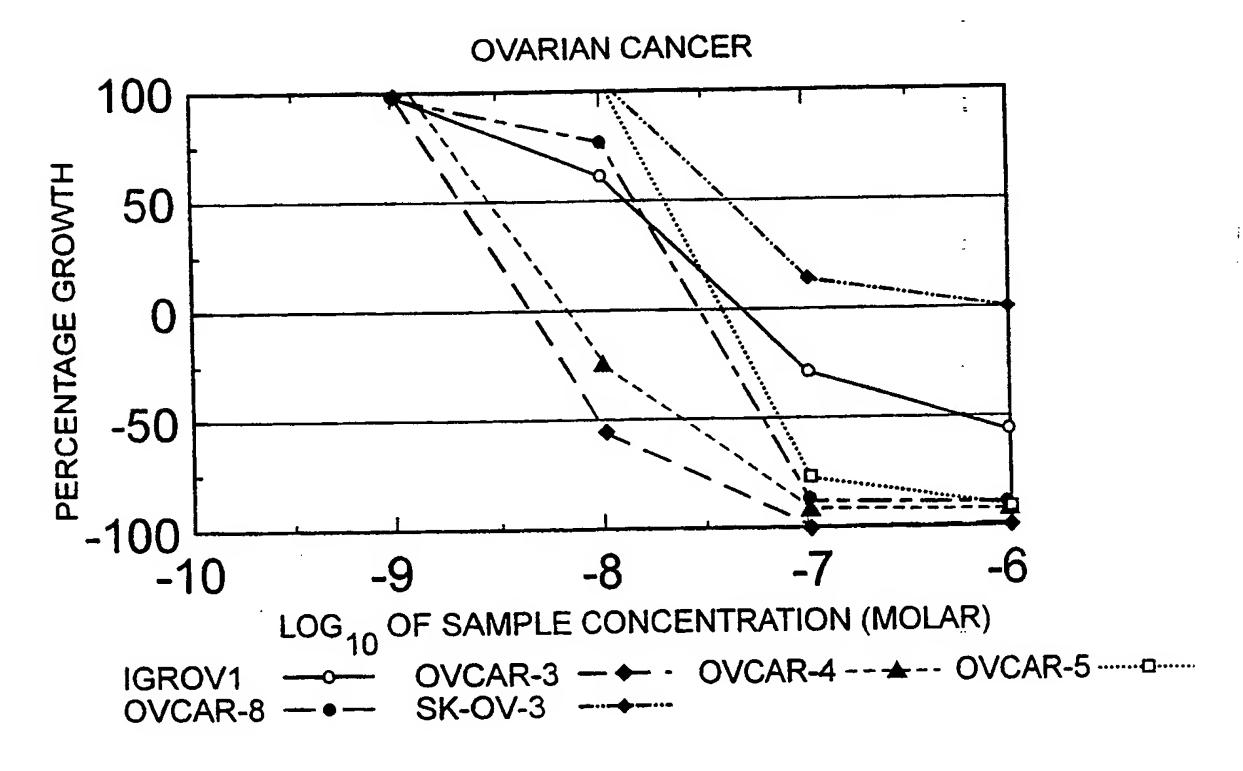
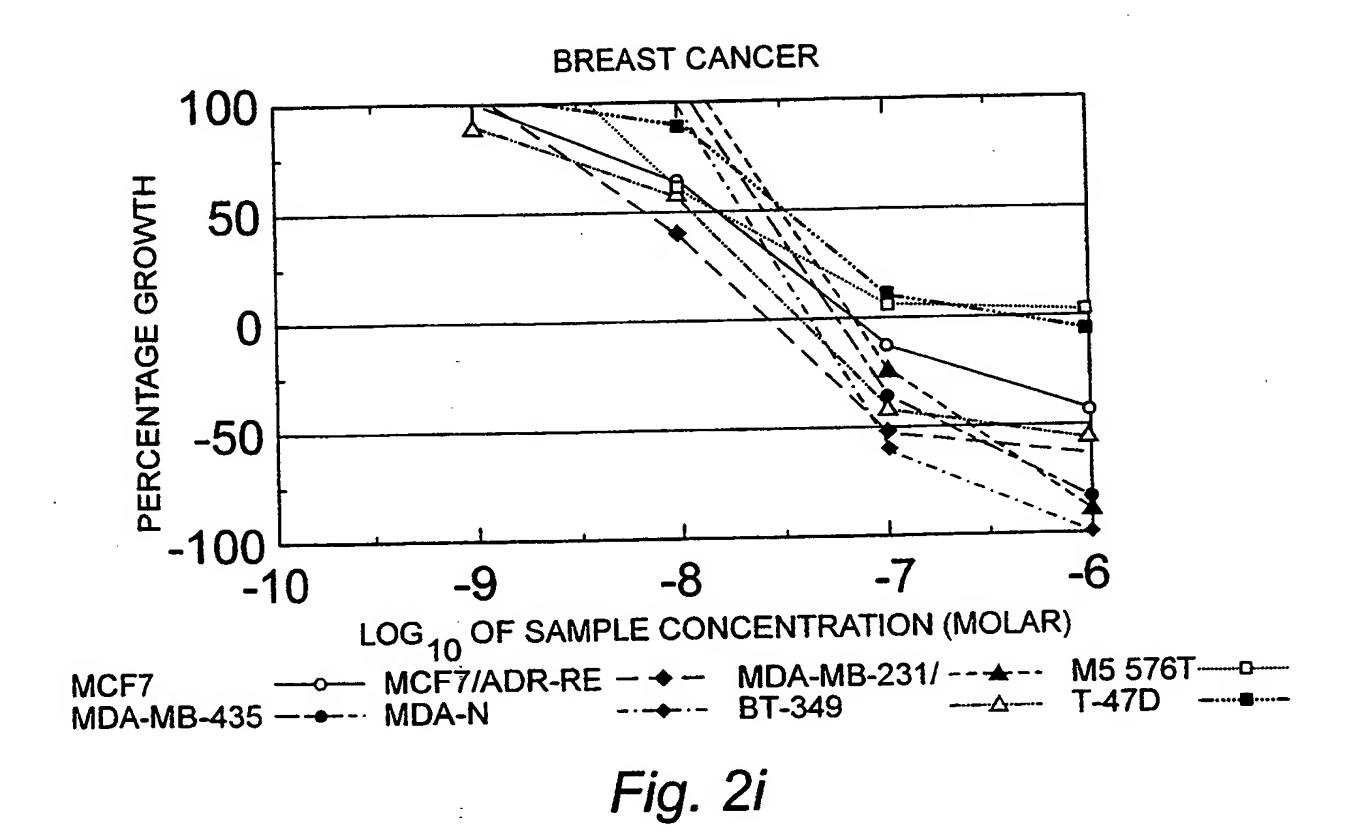


Fig. 2h



RECTIFIED SHEET (RULE 91)

National Cancer Institute Developmental Therapeutics Program	NSC: D-654033-O/0-2/2	0-2/2	Units: Molar SSPL:		Exp. ID: 9305MD56	SMD56	
Mean Graphs	Report Date: October 20, 1993	er 20,	Test Date: May 18, 19	993			
Panel/Cell Line	Log <sub>19</sub> GI50	G150	Log <sub>19</sub> TGI	TGI	Log <sub>19</sub> LC50	TCS0	
Leukemia							
CCRF-CEM	-8.42		-7.88		-7.06		
HL-60(TB)	-8.18		-7.68		-7.25		
K-562	-7.42		-7.03				
MOLT-4	-8.51		-7.90		-6.04		
RPMI-8226	-7.59		-7.24		-6.03		
SR	-8.34		-7.69		-7.12		
Non-small Cell Lung Cancer							
A549/ATCC	-8.29		-7.73		-7.31		
EKVX	-8.63		-7.75		-7.36		
HOP-62	-8.32		-7.79		-7.35		
HOP-92	-7.45		-7.04				
NCI-H226	-7.54		-7.16				
Fig. 2j	+3 +2 +1	0 -1 -2	-3 +2 +1 (	0 -1 -2	-3 +3 +2	2 +1 0 -1	1-2-3

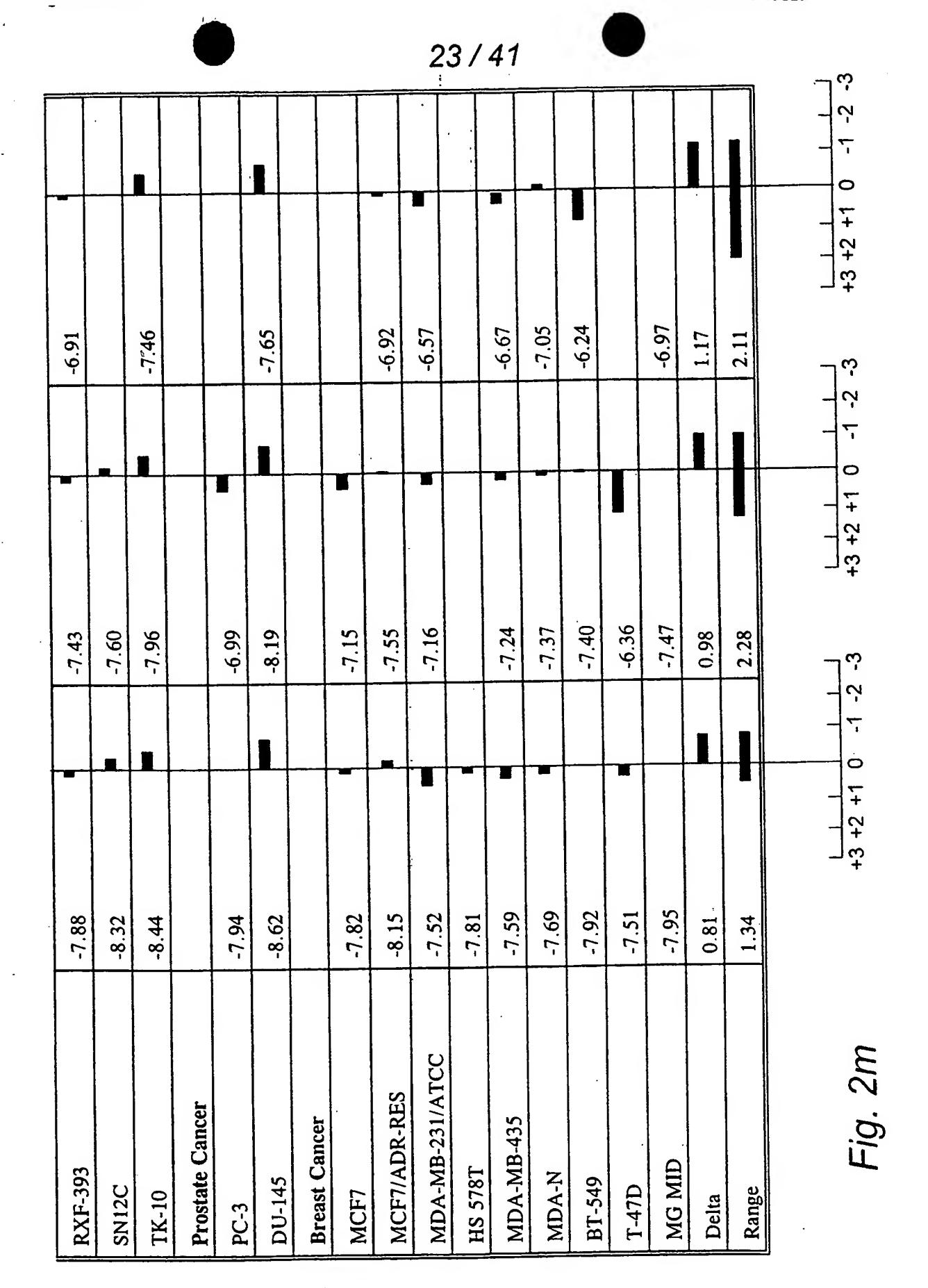
nncer ncer na	NCI-H23	-8.38	7.77	-7.24		
lterr	NCI-H460	-8.20	-7.65	-7.15		
DS -7.68 -7.39 -7.39 -7.39 -7.20 -7.56 -7.20 -7.77 -7.77 -7.77 -7.77 -7.77 -7.77 -7.77 -7.77 -7.77 -7.74 -7.74 -7.74 -7.74 -7.74 -7.74 -7.74 -7.74 -7.74 -7.74 -7.75 -7.74 -7.75 -7.74 -7.75 -7.74 -7.75 -7.	NCI-H522	-8.51	-7.91	-7.00		
38       -7.68       -7.39         18       -7.56       -7.20         2       -8.33       -7.77         2       -7.56       -7.17         3       -7.44       -7.24         10cer       -7.66       -7.24       -7.44         10cer       -8.06       -7.44       -7.44         10       -7.44       -7.49       -7.49         10       -7.74       -7.49       -7.49         10       -7.78       -7.24       -7.24         10       -7.58       -7.24       -7.49         10       -7.58       -7.24       -7.24         10       -7.58       -7.24       -7.24         10       -7.58       -7.24       -7.24         10       -7.58       -7.24       -7.24         10       -7.72       -7.45       -7.45         10       -7.72       -7.45       -7.45         10       -7.72       -7.45       -7.45         10       -7.72       -7.45       -7.45         10       -7.74       -7.44       -7.44       -7.44         10       -7.24       -7.24       -7.24       -7.24	Colon Cancer					1
38       -7.56       -7.77         5       -8.33       -7.17         1       -7.56       -7.14         1       -7.64       -7.24         1       -7.66       -7.24         1       -8.76       -8.45         1       -8.05       -7.44         1       -8.05       -7.44         1       -7.49       -7.49         1       -7.58       -7.24         1       -7.49       -7.49         1       -7.49       -7.49         1       -7.45       -7.45         1       -7.45       -7.45	COLO 205	-7.68	-7.39	-7.10		
5       -8.33       -7.77         -7.56       -7.17       -7.14         ncer       -7.64       -7.24         ncer       -8.76       -8.45         e.8.03       -7.44       -7.44         e.8.06       -7.49       -7.49         r.7.74       -7.49       -7.49         r. 3       -7.45       -7.45         r. 3       -7.45       -7.45         r. 3       -7.45       -7.45	HCC-2998	-7.56	-7.20	-6.54		3
-7.56       -7.17         -7.71       -7.44         ncer       -7.64       -7.31         ncer       -7.66       -7.24         s.76       -8.45       -8.45         -8.03       -7.44       -7.44         -8.06       -7.49       -7.49         -7.74       -7.24       -7.49         ma       -7.72       -7.45         ma       -7.72       -7.45	HCT-116	-8.33	7.77	-7.35		
ncer7.71 -7.44 -7.44 -7.66 -7.24 -7.24 -7.24 -7.24 -7.24 -8.76 -8.45 -8.45 -8.03 -7.44 -7.46 -7.24	HCT-15	-7.56	-7.17	-6.29		
ncer -7.64 -7.31 -7.34 -7.34 -7.24 -7.24 -8.76 -8.45 -8.45 -8.03 -7.44 -7.24 -7.24 -7.28 -7.28 -7.24 -7.28 -7.24 -7.28 -7.24 -7.23 -7.24 -7.32 -7.33 -7.34 -7.32 -7.33 -7.34 -7.32 -7.33 -7.34 -7.32 -7.33 -7.34 -7.34 -7.33 -7.34 -7.34 -7.33 -7.34 -7.34 -7.33 -7.34 -	HT29	-7.71	-7.44	-7.18		
ncer7.667.247.247.24	KM12 .	-7.64	-7.31	-6.91		2
ncer.       .8.76       -8.45       -8.45         -8.03       -7.44       -7.44         -8.06       -7.66       -7.49         -7.74       -7.49       -7.24         na       -7.72       -7.45	SW-620	-7.66	-7.24	-6.44		1/
-8.76       -8.45         -8.03       -7.44         -8.06       -7.66         -7.74       -7.49         -7.58       -7.24         -7.72       -7.45         -7.45       -7.45         -7.72       -7.45	CNS Cancer					41
-8.03 -7.44 -7.44 -7.66 -8.06 -7.74 -7.24 -7.28 -7.24 -7.25 -7.72 -7.45	SF-268	-8.76	-8.45	-8.14		
-8.06       -7.66         -7.74       -7.49         -7.58       -7.24         -7.72       -7.45         ma       -7.45	SF-295	-8.03	-7.44	-6.46		
-7.74       -7.49         -7.58       -7.24         -7.72       -7.45         ma       -7.45	SF-539	-8.06	-7.66	-7.29	•	
-7.58 -7.24 -7.24 -7.45 ma	SNB-19	-7.74	-7.49	-7.25		
na -7.72 -7.45	SNB-75	-7.58	-7.24	-6.50		
Melanoma	U251	-7.72	-7.45	-7.19		
	Melanoma					

RECTIFIED SHEET (RULE 91)

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LOX IMVI	-7.70	-7.40	-7.11	
MALME-3M	-7.76	-7.45	-7.13	
M14	-7.57	-7.22	-6.20	
SK-MEL-2	-7.66	-7.31	-6.71	
SK-MEL-28	-7.80	-7.35	-6.47	
SK-MEL-5	-7.89	-7.59	-7.29	
UACC-257	-7.61	-7.28		
UACC-62	-7.58	-7.21	-6.38	
Ovarian Cancer				
IGROVI	-7.90	-7.34	-6.31	
OVCAR-3	-8.68	-8.36	-8.04	
OVCAR-4	-8.55	-8.19	-7.63	
OVCAR-5	-7.70	-7.43	-7.15	
OVCAR-8	-7.85	-7.54	-7.23	
SK-0V-3	-7.42	-6.17		
Renal Cancer				
0-982	-8.47	-7.89	-7.17	
ACHN	-8.36	-7.52		
CAK1-1	-7.51	-7.04		



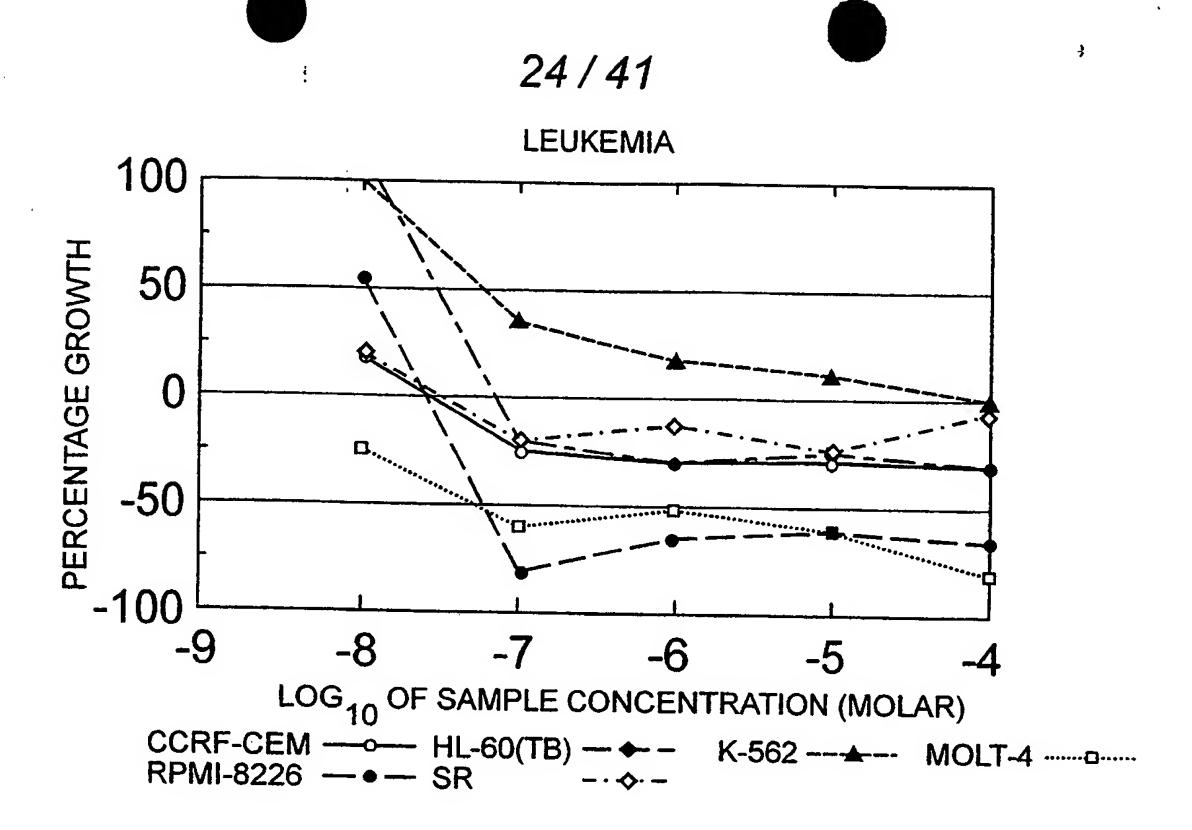


Fig. 3a

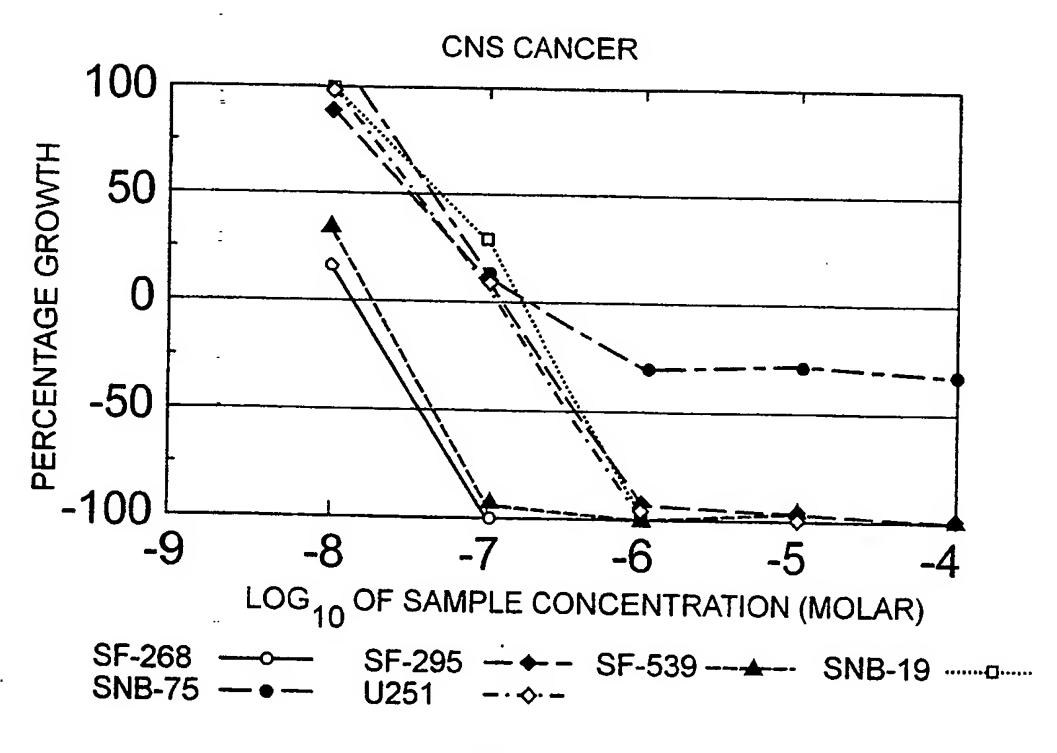


Fig. 3b

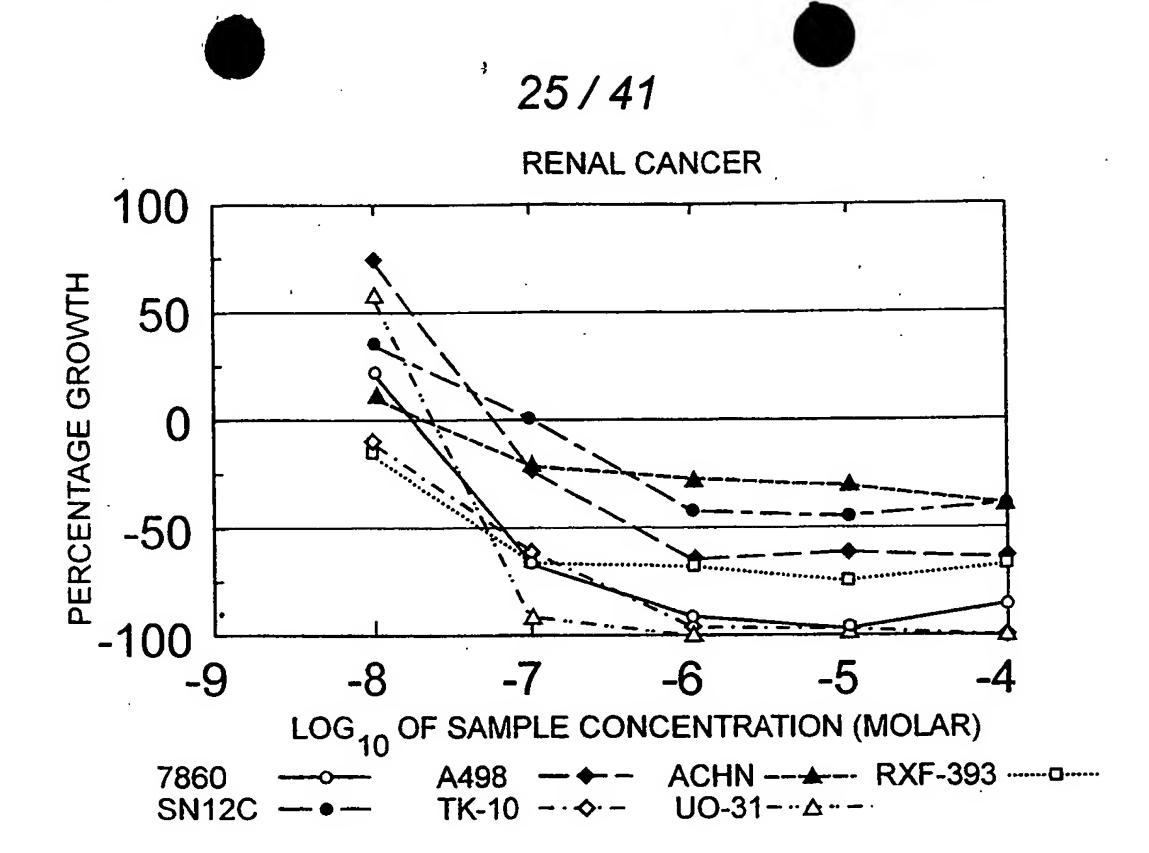
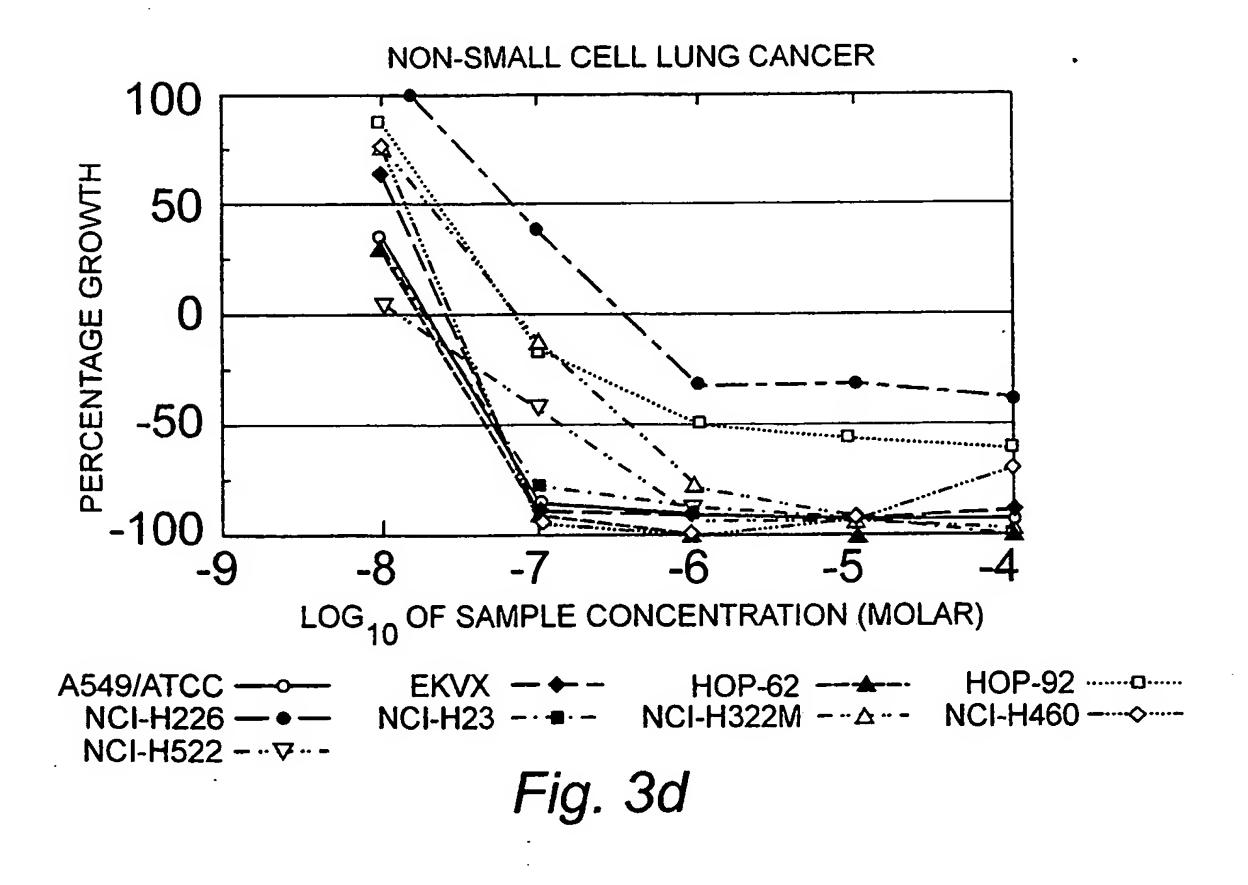


Fig. 3c



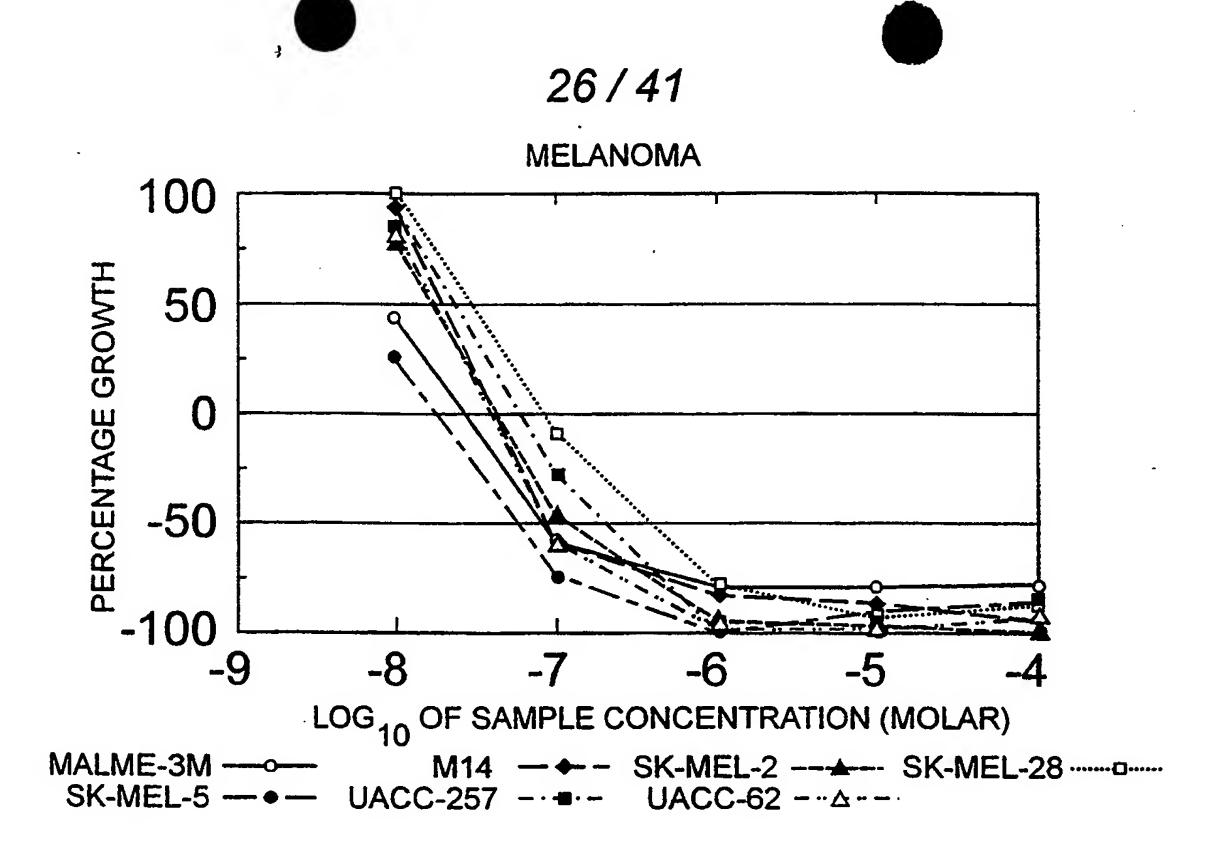


Fig. 3e

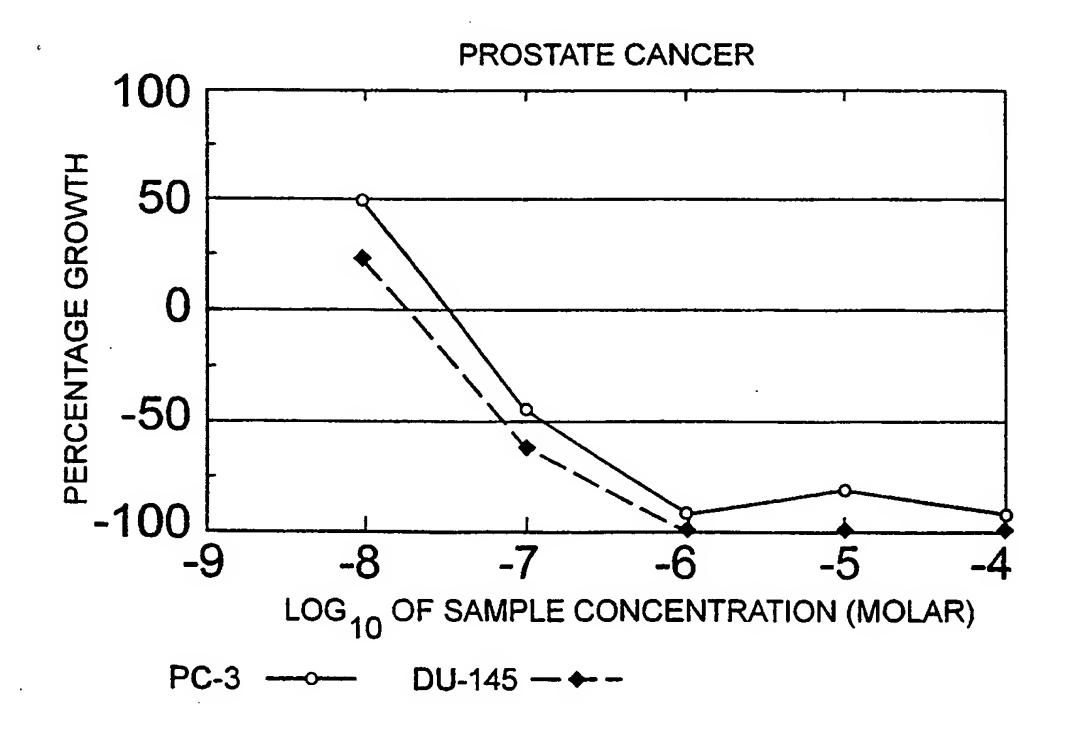


Fig. 3f

DED COMPRESSORS (CERTS) AND STREET TO A A ...

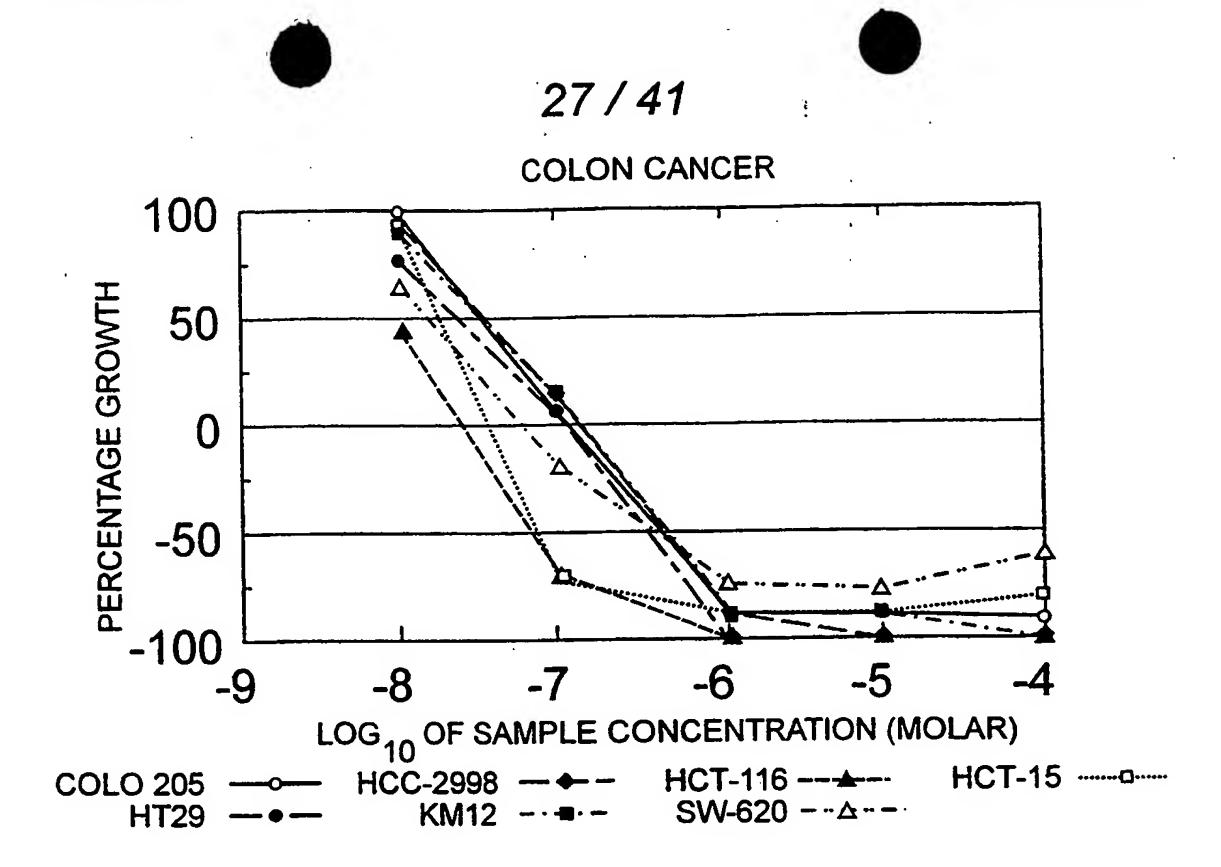
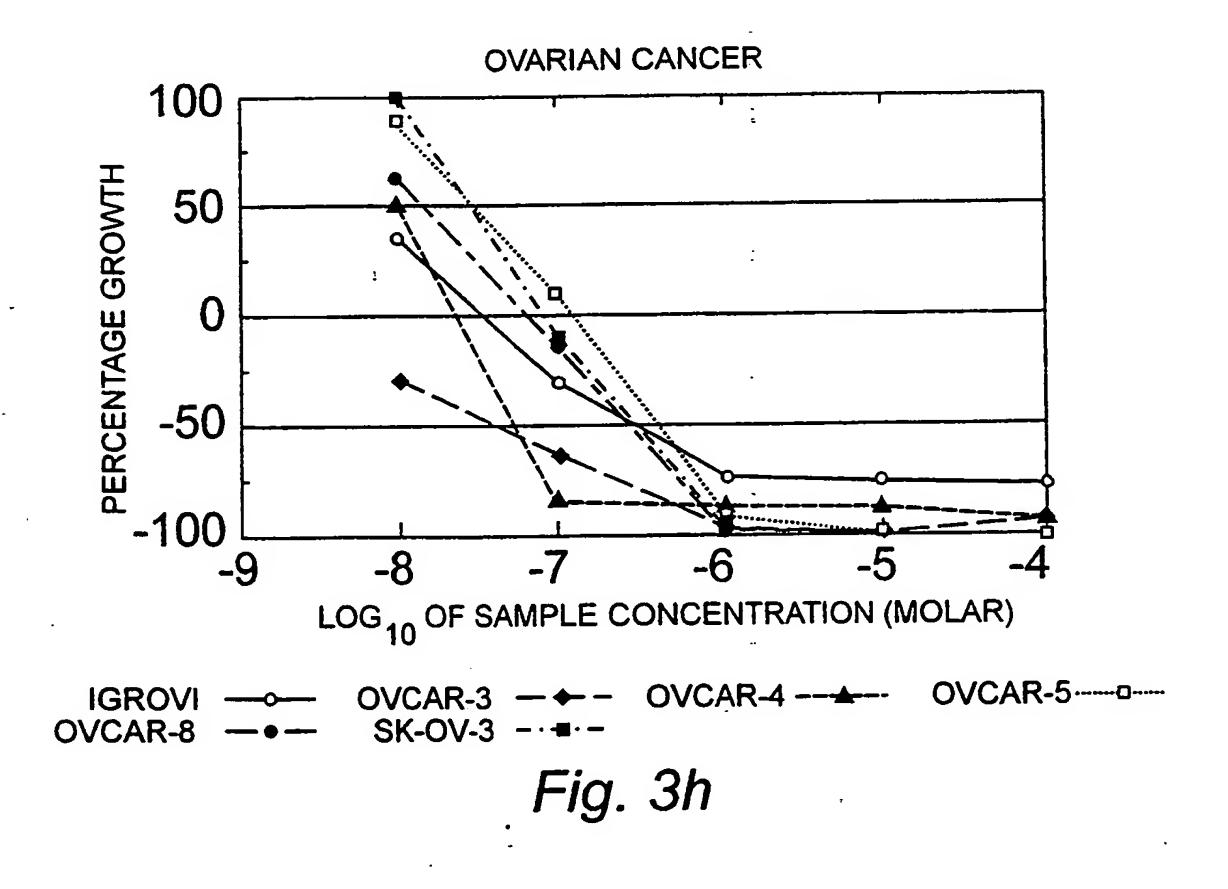


Fig. 3g



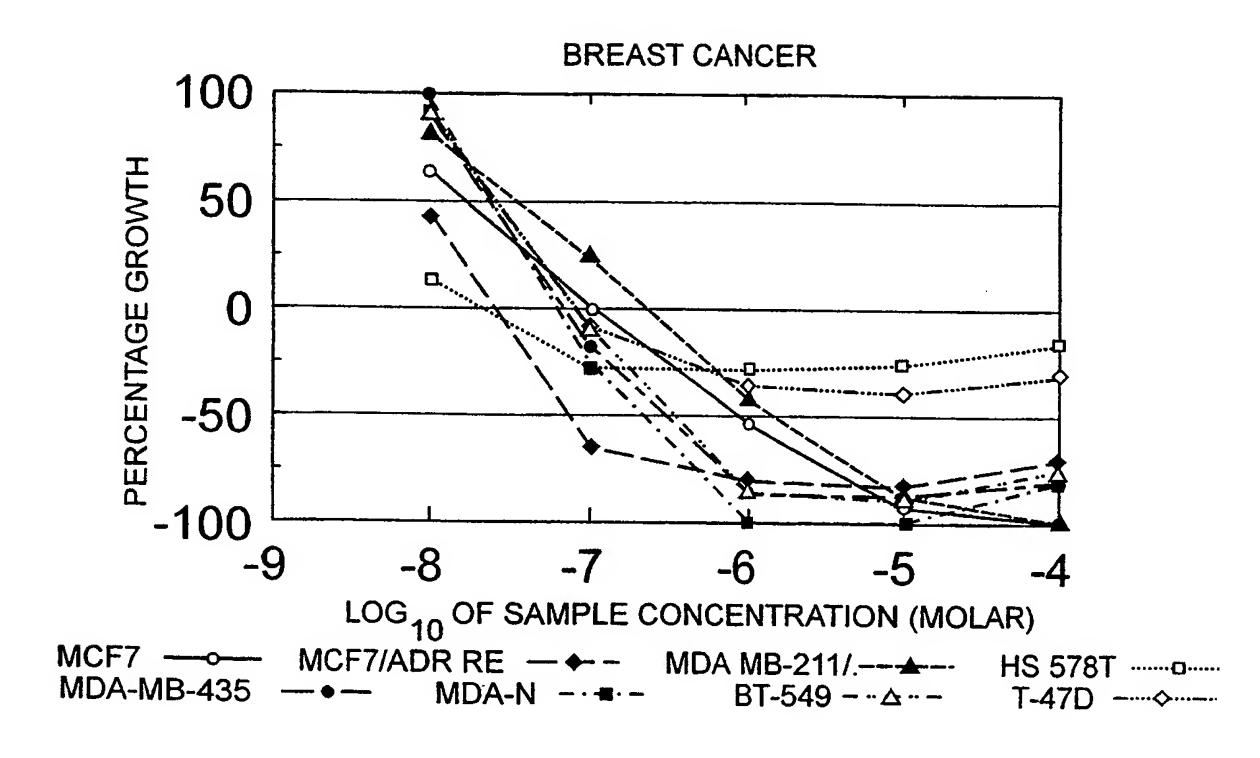


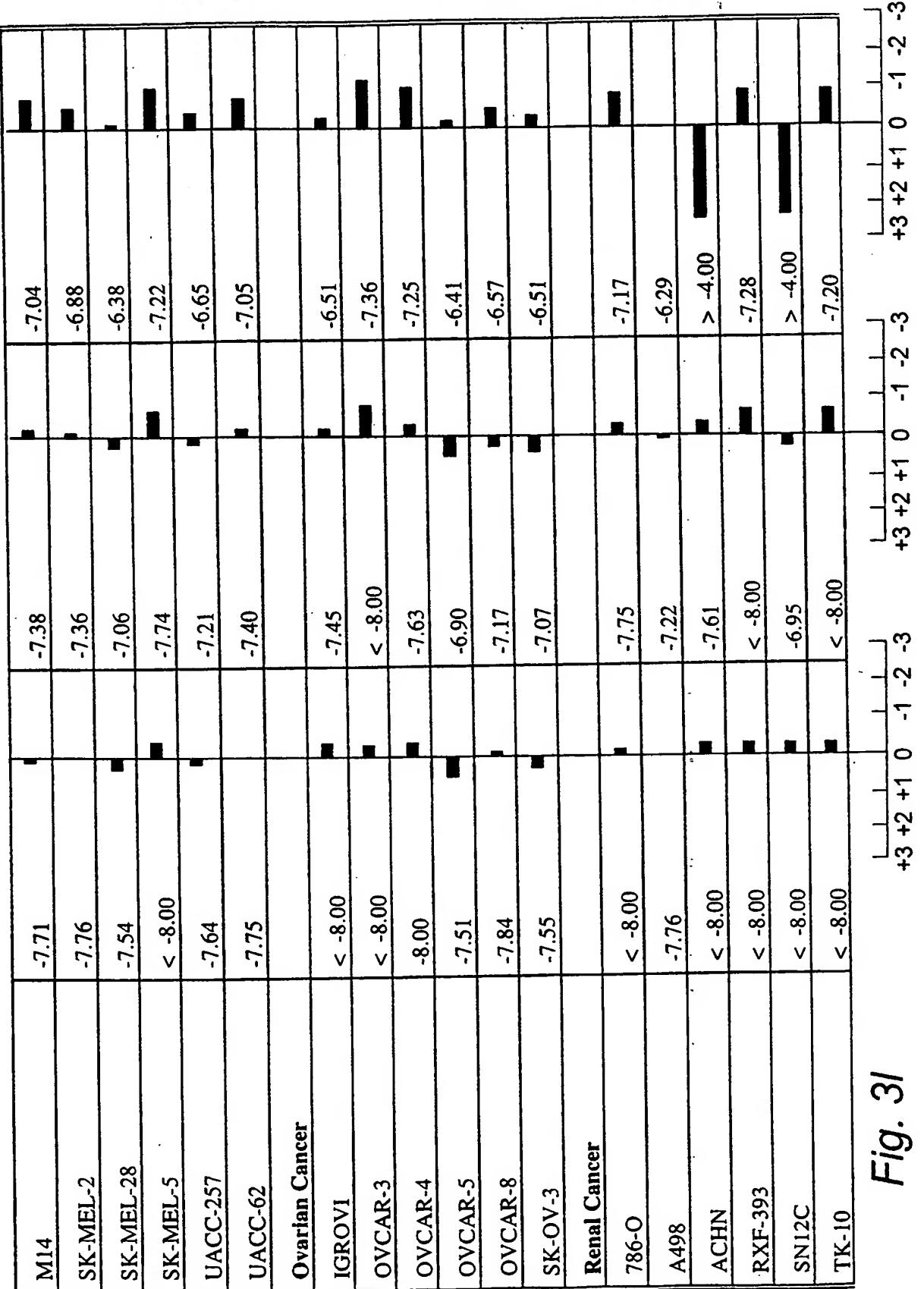
Fig. 3i

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National Cancer Institute Developmental Therapeutics Program	NSC: D-659471-Y/0-1/5	Units: Molar SSPL:	Exp. ID: 9302MD33
Mean Graphs - Uscharidin	Report Date: March 8, 1993	Test Date: February 23, 1993	
Panel/Cell Line	Log GI50 G150	Log <sub>19</sub> TGI TGI	Log <sub>19</sub> LC50 LC50
Leukemia			
CCRF-CEM	< -8.00	-7.64	> -4.00
HL-60(TB)	-7.97	-7.59	-7.22
K-562	-7.25	> -4.00	> -4.00
MOLT-4	< -8.00	< -8.00	-7.35
RPMI-8226	-7.51	-7.15	> -4.00
SR	< -8.00	-7.55	> -4.00
Non-small Cell Lung Cancer			
A549/ATCC	< -8.00	-7.71	-7.29
EKVX	-7.91	-7.58	-7.25
HOP-62	8.00	-7.74	7.32
HOP-92	-7.65	-7.13	5.77
NCI-H226	-7.15	-6.44	> -4.00
NCI-H23	< -8.00	-7.74	-7.27
Fig. 3j	+3 +2 +1 0 -1 -2	2 -3 +3 +2 +1 0 -1 -2	-3 +3 +2 +1 0 -1 -

30/41

NCI-H322M	-7.71	-7.10		-6.39	
NCI-H460	-7.83	-7.55		-7.26	
NCI-H522	< -8.00	-7.87		-6.83	
Colon Cancer					
COLO 205	-7.49	-6.94		-6.40	
HCC-2998	-7.45	-6.85		-6.37	
HCT-116	> -8.00	-7.61		-7.16	
HCT-15	-7.76	-7.45		-7.14	
HT29	-7.64	-6.94		-6.47	
KM12	-7.49	-6.86		-6.36	
SW-620	-7.84	-7.23		-6.44	
CNS Cancer					
SF-268	< -8.00	-7.87		-7.43	
SF-295	-7.52	-6.91		-6.41	
SF-539	< -8.00	-7.74		-7.34	
SNB-19	-7.32	-6.79		-6.39	
SNB-75	-7.37	-6.69		> -4.00	
U251	-7.47	-6.95		-6.48	
Melanoma					
MALME-3M	< -8.00	-7.57		-7.07	
Fig. 3k	+3 +2 +1 0	-1 -2 -3	+3 +2 +1 0 -1 -2	-3 +3 +2 +1 0	1 -2 -3



UO-31	-7.96	-7.62		-7.27		
Prostate Cancer						
PC-3	< -8.00	-7.48		-6.87		
DU-145	< -8.00	-7.73		-7.15		
Breast Cancer						
MCF7	-7.78	-6.96		-6.03		
MCF7/ADR-RES	< -8.00	-7.59		-7.13		
MDA-MB-231/ATCC	-7.47	-6.63		-5.79		
11S 578T	< -8.00	-7.66		> -4.00		
MDA-MR-435	-7.63	-7.16		-6.52		3
MDAN	-7.62	-7.21		-6.67	<b>1</b>	32 /
RT 540	-7.71	-7.12		-6.46		41
T-47D	-7.62	-7.10		> -4.00		
MG MID	-7.78	-7.30		-6.33		
Delta	0.22	0.70		1.11		
Range	0.85	4.00		3.43		
Fig. 3m	+3 +2 +1 0	-1 -2 -3	+3 +2 +1 0 -1 -	-2 -3 +3 +2 +1	0 -1 -2 -	— რ

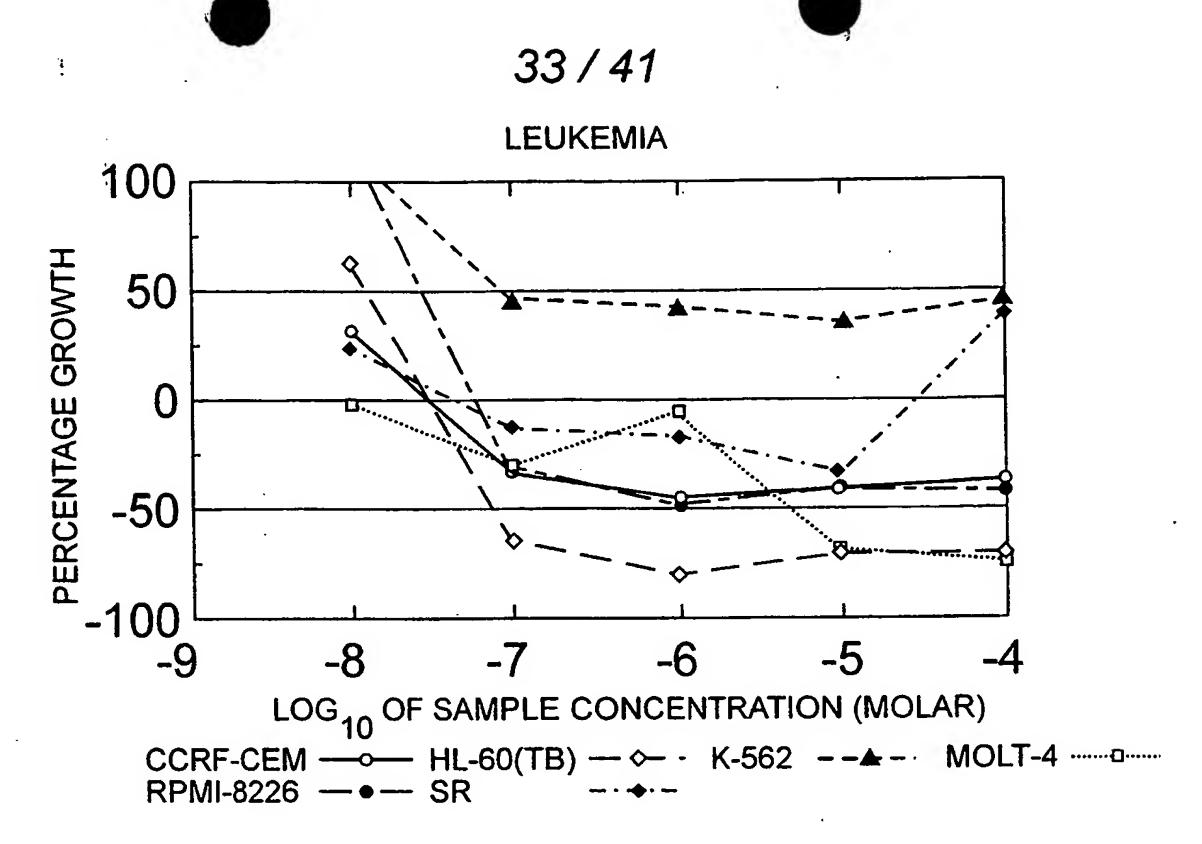


Fig. 4a

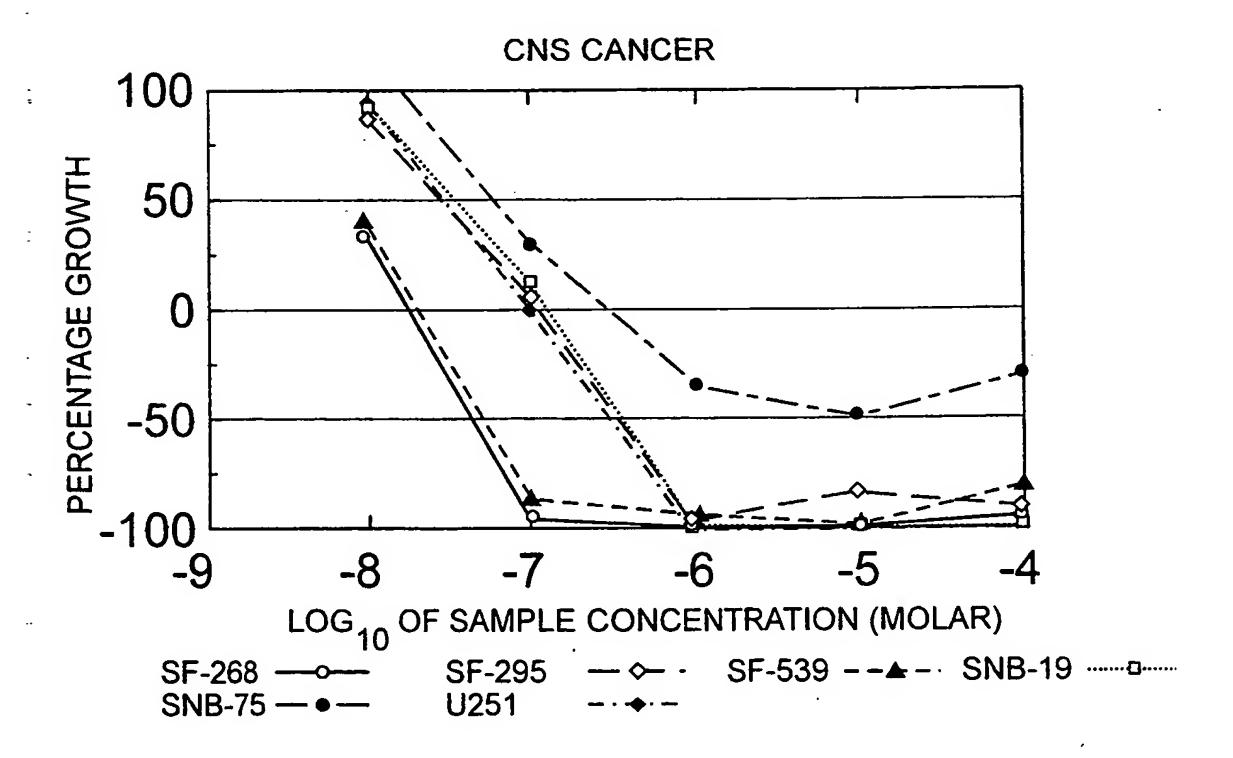


Fig. 4b

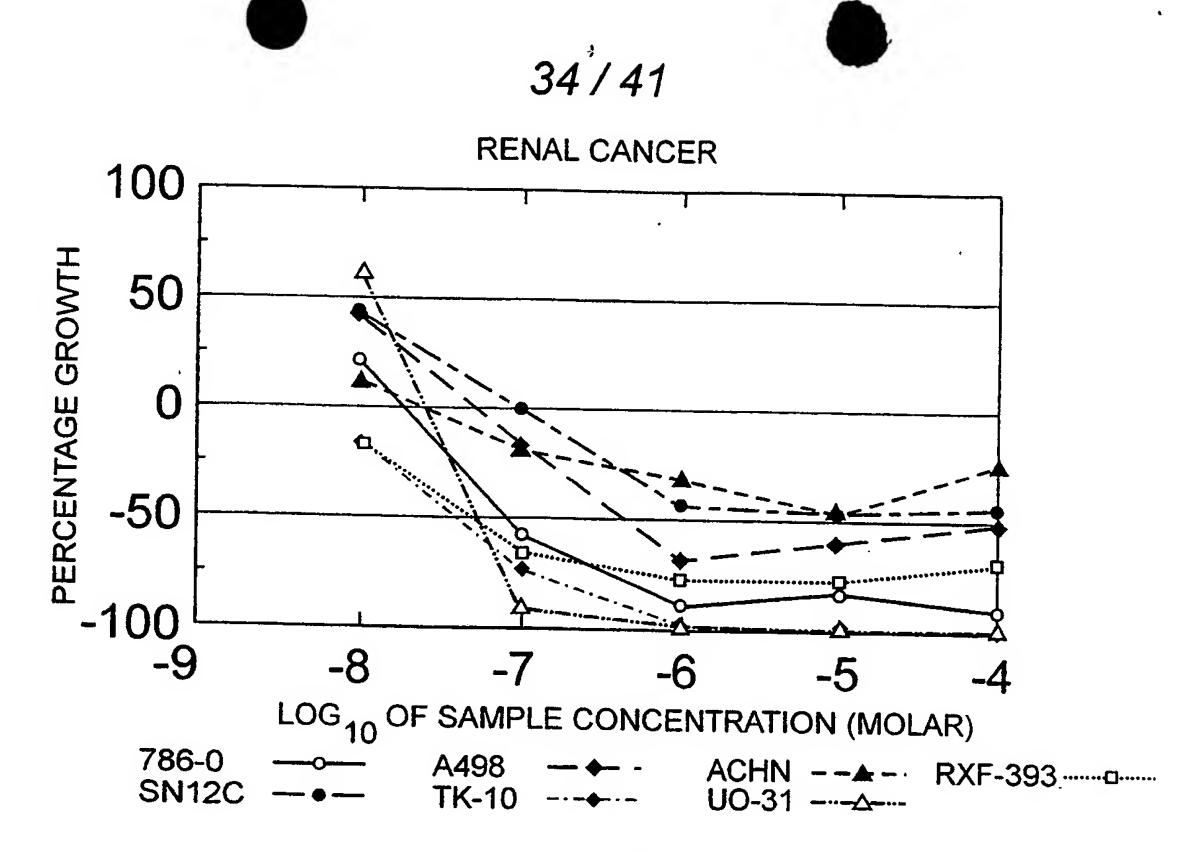


Fig. 4c

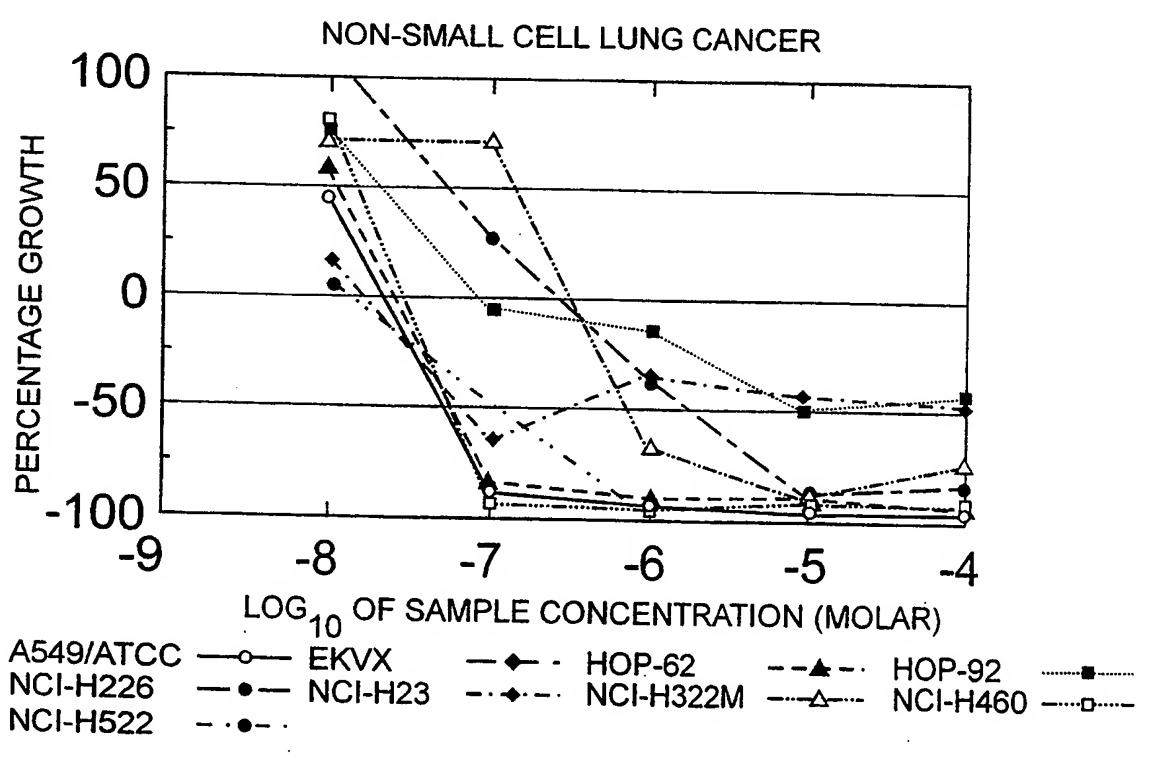


Fig. 4d

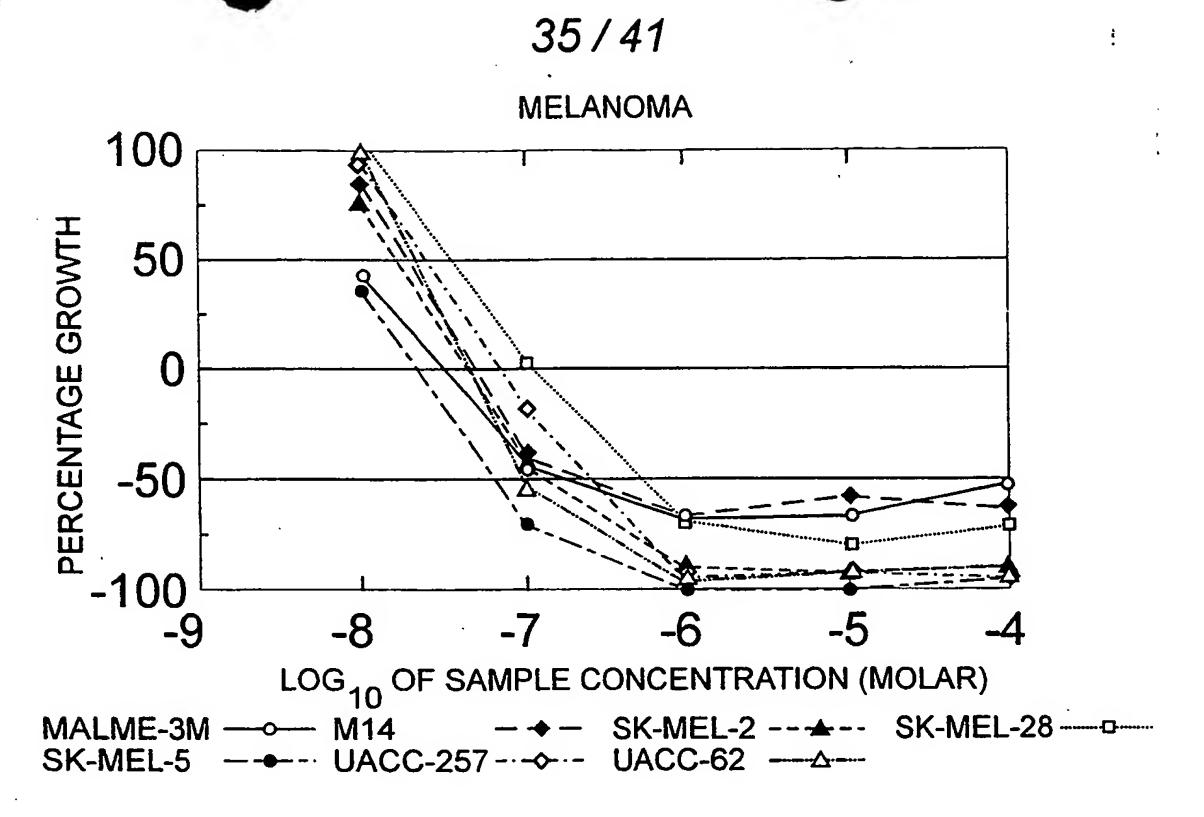


Fig. 4e

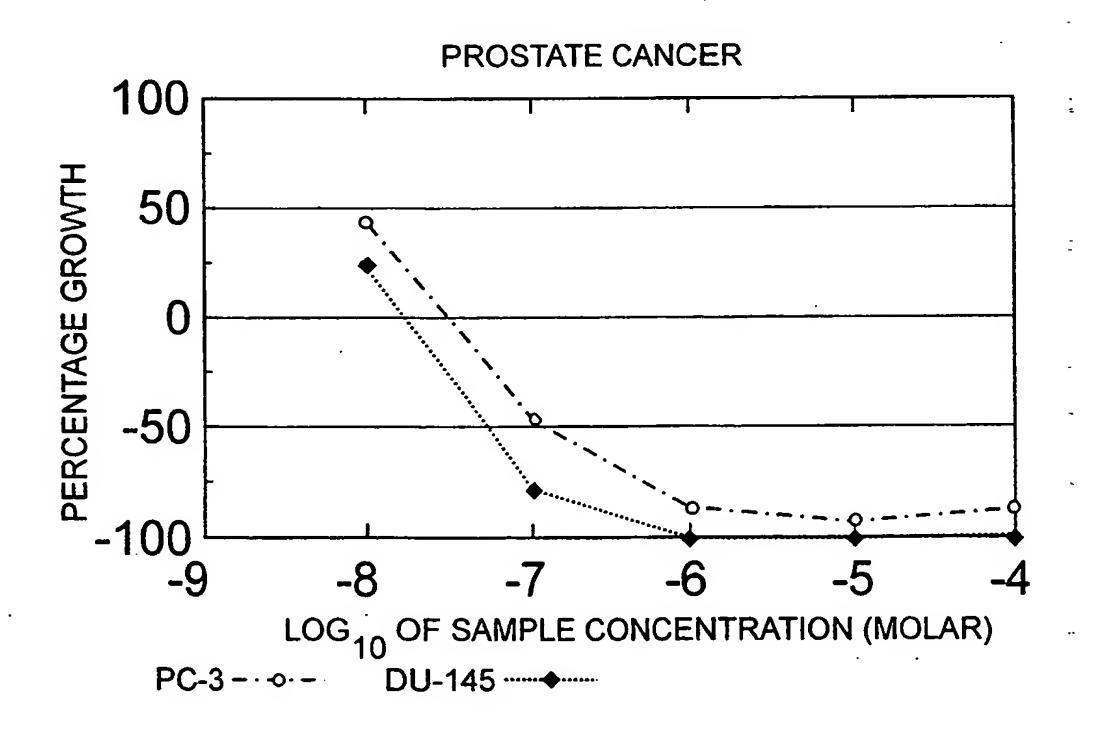


Fig. 4f

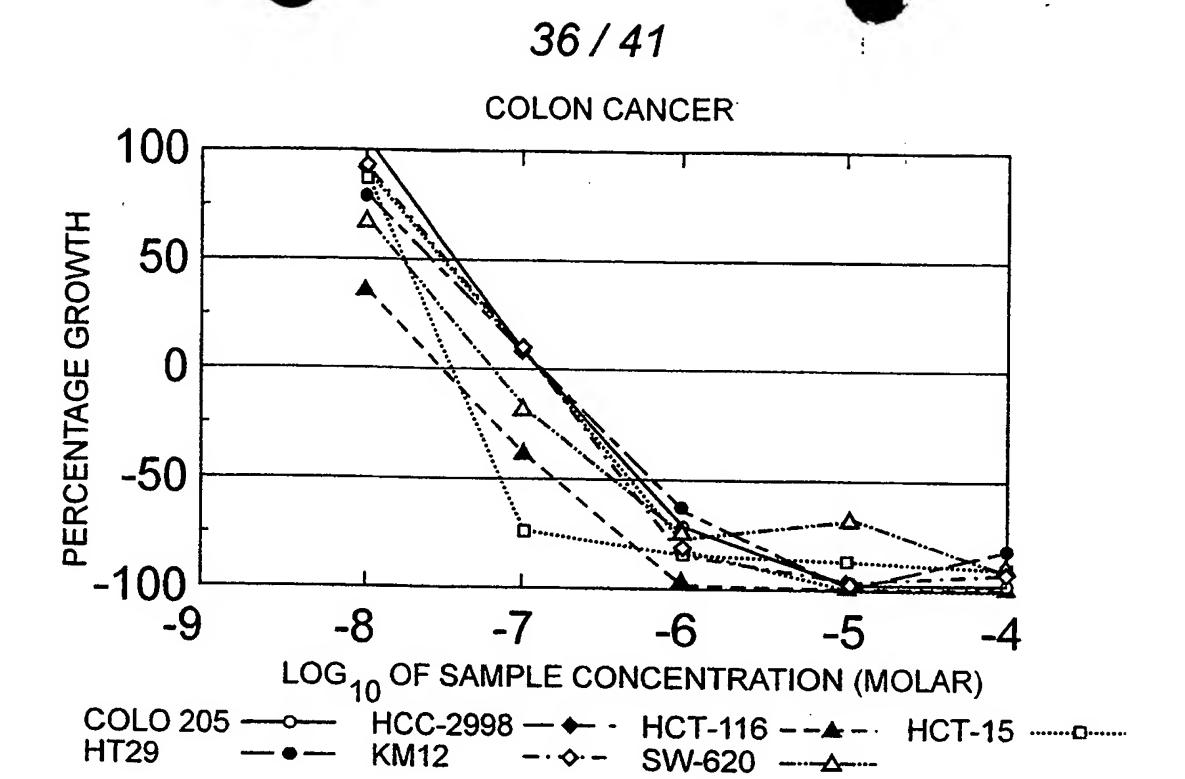


Fig. 4g

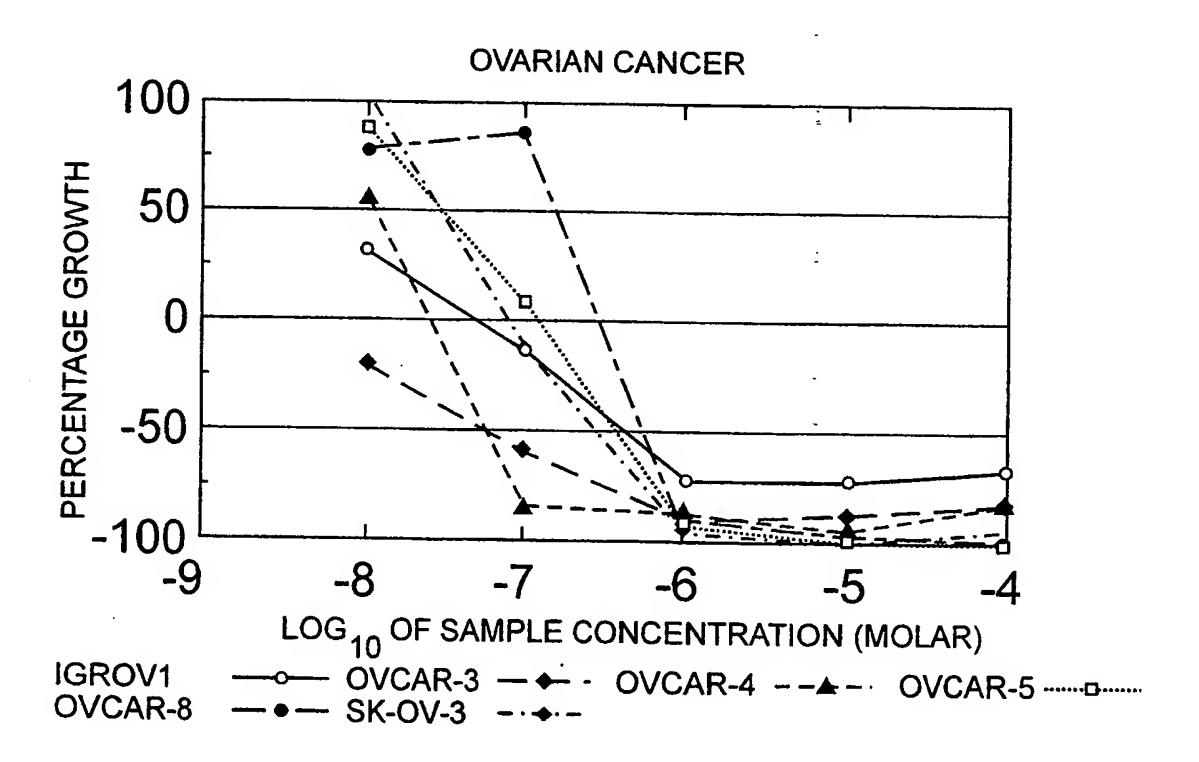
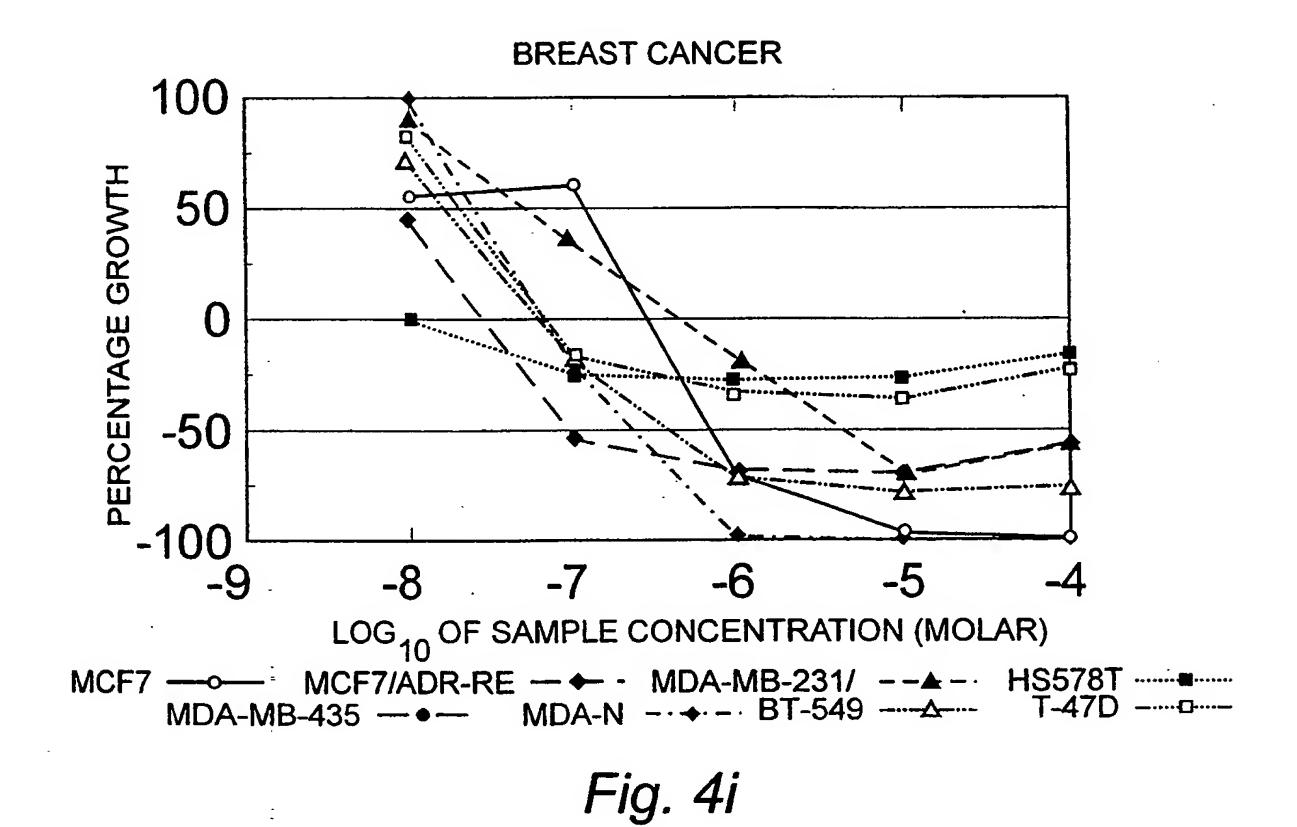


Fig. 4h



RECTIFIED SHEET (RULE 91)

National Cancer Institute Developmental Therapeutics Program	NSC: D-659472-Z/0-1/16	-1/16	Units: Molar SSPL:		Exp. ID: 9302MD33	
Mean Graphs - Calotoxin	Report Date: March 8, 1993	h 8, 1993	Test Date: February	23, 1993		
Panel/Cell Line	Log <sub>19</sub> GIS0 G	G150	Log19 TGI TGI		Log <sub>19</sub> LC50 LC50	20
Leukemia						
CCRF-CEM	< -8.00		-7.51		> -4.00	
HL-60(TB)	-7.93		-7.52		-7.10	
K-562	-7.08		> -4.00		> -4.00	·
MOLT-4	< -8.00		< -8.00		-5.28	
RPMI-8226	-7.57		-7.22		> -4.00	
SR	< -8.00				> -4.00	
Non-small Cell Lung Cancer						
A549/ATCC	< -8.00	-	-7.67		-7.29	
EKVX	-7.95		-7.59		-7.22	
HOP-62	-7.95		-7.60		-7.25	
HOP-92	-7.69		-7.07		> -4.00	
NCI-H226	-7.28		-6.57		> 4.00	
Fig. 4j	+3 +2 +1	0 -1 -2 -	3 +2 +1 0	-1-2	3 +3 +2 +1 0	-1 -2 -3

NCI-H23	< -8.00	-7.78		
NCI-H322M	-6.86	-6.49	-6.13	Ī
NCI-H460	-7.83	-7.54	-7.25	
NCI-H522	< -8.00	-7.85	-6.92	
Colon Cancer				
COLO 205	-7.46	-6.95	-6.28	
HCC-2998	-7.52	-6.92	-6.38	
HCT-116	< -8.00	-7.52	-6.80	
HCT-15	-7.79	-7.47	-7.15	
HT29	-7.63	-6.94	-6.21	
KM12	-7.55	-6.94	-6.35	
SW-620	-7.82	-7.22	-6.45	
CNS Cancer				
SF-268	< -8.00	-7.72	-7.34	
SF-295	-7.50	-68.9	-6.40	
SF-539	8.00	-7.67	-7.28	T
SNB-19	-7.42	-6.84	-6.41	
SNB-75	-7.18	-6.48	> -4.00	T
U251	-7.50	-6.95	-6.48	•
Fig. 4k	+3 +2 +1 0 -1 -2	-3 +3 +2 +1 0 -1 -2	-3 +3 +2 +1 0 -1	-2 -3

-8.00       -7.51       -6.55         -7.81       -6.98       -6.90         -7.48       -6.98       -6.27         -7.48       -6.98       -6.27         -7.63       -7.67       -7.19         -7.63       -7.35       -7.19         -8.00       -7.35       -7.20         -7.53       -6.93       -6.42         -6.53       -6.53       -6.53         -6.53       -6.53       -6.53         -8.00       -7.74       -6.53         -8.00       -7.74       -6.53         -8.00       -7.74       -6.53         -8.00       -7.74       -6.53         -8.00       -7.74       -6.53         -8.00       -7.74       -6.53         -8.00       -7.74       -6.53         -8.00       -7.74       -6.53         -8.00       -7.74       -6.53         -8.00       -7.74       -6.53         -8.00       -8.00       -7.74         -8.00       -8.00       -7.74         -8.00       -8.00       -7.59         -8.00       -7.79       -7.59         -8.00       -7.79       -7.59	Melanoma					
-7.73       -7.33       -6.65         -28       -7.48       -6.98       -6.27         -8       -7.67       -6.27       -7.19         -5       -7.63       -7.67       -6.27         -7.63       -7.67       -6.60       -7.19         -7.69       -7.35       -7.23       -6.60         3       -8.00       -7.31       -6.35       -6.42         4       -7.96       -7.50       -7.50       -7.23         8       -6.81       -6.53       -6.42         8       -6.81       -6.53       -6.25         9       -7.55       -7.11       -7.78         108       -8.00       -7.74       -7.08         108       -8.00       -7.27       -6.32         108       -8.00       -7.27       -7.08         108       -8.00       -7.27       -7.40         108       -8.00       -7.27       -7.40         108       -8.00       -7.27       -7.40         109       -7.27       -7.40       -7.40         109       -7.27       -7.40       -7.40         109       -7.27       -7.40       -7.40	MALME-3M		-7.51	-6.75		
2       7.81       7.38       6.98       6.527         28       -6.27       -6.27       -6.27         -5       -6.27       -7.19       -6.527         57       -7.63       -7.18       -6.60         57       -7.63       -7.18       -6.60         2       -7.69       -7.31       -7.02         3       -8.00       -7.50       -7.50       -7.23         4       -7.96       -6.53       -6.42       -6.42         8       -6.81       -6.53       -6.42       -6.42         8       -6.81       -6.53       -6.25       -6.25         9       -7.55       -7.11       -6.53       -6.25         9       -8.00       -7.74       -6.53       -6.25         9       -8.00       -7.74       -6.53       -6.25         10       -7.55       -7.11       -7.12       -7.08         10       -8.00       -7.74       -7.08       -7.08         10       -8.00       -7.27       -7.08       -7.08         10       -8.00       -7.27       -7.08       -7.08         10       -8.00       -7.27       -7.08	M14	-7.73	-7.33	-6.65		
-58       -6.98       -6.98       -6.27         -5       -8.00       -7.67       -7.19       -7.19         57       -7.63       -7.18       -7.19       -7.10         2       -7.69       -7.35       -7.02         2       -8.00       -7.31       -7.20         3       -8.00       -7.60       -7.23         4       -7.96       -7.60       -7.23         8       -6.81       -6.53       -6.42         8       -6.81       -6.53       -6.53         9       -7.74       -6.53         9       -7.74       -6.53         9       -8.00       -7.74       -6.53         8       -8.800       -7.74       -6.53         8       -8.800       -7.74       -6.53         8       -8.800       -7.74       -6.53         8       -8.800       -7.74       -6.53         8       -8.800       -7.74       -6.53         8       -8.800       -7.29       -7.20         9       -8.800       -7.29       -7.29         10       -7.31       -7.32         10       -7.53       -7.53	SK-MEL-2	-7.81	-7.38	06.9-		
-5       < -8.00	SK-MEL-28	-7.48	-6.98	-6.27		
57       -7.63       -7.18       -6.60         2       -7.69       -7.35       -6.50         Cancer       -8.00       -7.31       -6.36         3       -8.00       -7.31       -6.36         4       -7.96       -7.60       -7.23         5       -7.53       -6.93       -6.42         8       -6.81       -6.53       -6.25         8       -6.81       -6.53       -6.25         9       -7.74       -7.74       -6.53         9       -8.00       -7.74       -7.74       -6.32         8       -8.00       -7.77       -7.27       -6.32         9       -8.00       -7.74       -7.74       -7.08         1       -7.75       -7.27       -6.32       -7.08	SK-MEL-5	< -8.00	19.7-	-7.19		
Cancer       -7.69       -7.35       -7.02         Cancer       -8.00       -7.31       -6.36         3       <-8.00       -7.60       -7.20         4       -7.96       -7.60       -7.20         5       -7.53       -6.53       -6.42         8       -6.81       -6.53       -6.53         3       -6.53       -6.53       -6.53         3       -7.55       -7.11       -6.53         3       -8.00       -7.74       -6.53         4       -7.74       -6.53       -6.53         5       -8.00       -7.74       -6.53         6       -7.27       -6.53       -6.53         8       -6.53       -6.53       -6.53         9       -6.53       -6.53       -6.53         10       -7.74       -6.53       -6.53         8       -8.00       -7.74       -6.53       -6.53         8       -8.00       -7.27       -7.27       -6.53         8       -8.00       -7.27       -7.27       -7.29       -7.29         9       -8.00       -7.27       -7.27       -7.27       -7.27       -7.27	UACC-257	-7.63	-7.18	-6.60		
Cancer       Cancer       -3.31       -6.36         3       < -8.00	UACC-62	-7.69	-7.35	-7.02		
3       < -8.00	Ovarian Cancer					
3       < -8.00	IGROVI		-7.31	-6.36		4
1.96       -7.60       -7.23       -6.93       -6.42         1.53       -6.53       -6.25       -6.25         1.25       -7.11       -6.53       -6.53         1.02       -7.11       -6.53       -6.53         1.02       -7.11       -6.53         1.02       -7.74       -6.53         1.03       -7.27       -7.08         1.11       -7.11       -7.40         1.11       -7.59       -7.40	OVCAR-3			-7.20		0/
-6.81       -6.93       -6.42         ncer       -6.53       -6.25         ncer       -7.11       -6.53       -6.53         ncer       -7.14       -6.53         c -8.00       -7.74       -7.08         c -8.00       -7.27       -6.32         c -8.00       -7.27       -6.32         c -8.00       -7.27       -6.32	OVCAR-4	<i>-7.96</i>	-7.60	-7.23		41
1.55	OVCAR-5	-7.53	-6.93	-6.42		
ncer       -7.55       -7.11       -6.53         ncer       -7.74       -7.74       -7.08         c -8.00       -7.27       -6.32         c -8.00       -7.27       -6.32         c -8.00       -7.59       -6.32         c -8.00       -7.59       -6.32	OVCAR-8	-6.81	-6.53	-6.25		
C -8.00   -7.74   -7.08   -7.08   -7.27   -6.32   -6.32   -6.32   -7.59   -7.59   -7.60   -7.59   -7.60   -7	SK-0V-3	-7.55	-7.11	-6.53		
< -8.00	Renal Cancer					
4       -8.00       ■       -7.27       -6.32         4       < -8.00	0-982		-7.74	-7.08		
< -8.00	A498		-	-6.32		į.
	ACHN		-7.59			
+2 +4 0 -4 -2 -3 +3 +2 +1 0 -4 -2 -3	Ein III	- 7		+1 0 -1 -2 -	+2 +1 0 -1 -2	<b>-</b> ب

RXF-393	< -8.00	< -8.00	-7.28		
SN12C	< -8.00	-7.00	> -4.00		
TK-10	< -8.00	> -8.00	-7.36		
UO-31	-7.93	-7.61	-7.29		1
Prostate Cancer					
PC-3	< -8.00	-7.49	-6.85		
DU-145	< -8.00	-7.74	-7.25		
Breast Cancer					· ·
MCF7	-6.94	-6.55	-6.16		
MCF7/ADR-RES	< -8.00	-7.54	-7.03		4
MDA-MB-231/ATCC	-7.29	-6.34	-5.35		1/
HS 578T	< -8.00	-7.96	> -4.00		41
MDA-MB-435	-7.67	-7.15	-6.38		
MDA-N	-7.60	-7.17	-6.62		
BT-549	-7.76	-7.20	-6.39		
T47D	-7.65	-7.16	> 4.00		)
MG MID	-7.73	-7.25	-6.19		
Delta	0.27	0.75	1.17		
Range	1.19	4.00	3.36		
Fig. 4m	+3 +2 +1 0 -1	1 -2 -3 +3 +2 +1 0	1 -2 -3 +3 +2 +1	0 -1 -2 -	– ფ

### INTERNATIONAL SEARCH REPORT

Int tional Application No PCT, 8/01522

A. CLA	SSIFICATION OF SUBJECT MALE	
IPC	VC1/CL SUBJECT WHITE	l
11 C	A61K31/365	

According to International Patent Classification (IPC) or to both national classification and IPC

#### B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 6 A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

Category *	Citation of document, with indication, where appropriate, of the relevant passages	
	where appropriate, of the relevant passages	Relevant to claim No.
X	J.A. PARSONS: "Cat assay for the emetic action of digitalis and elated glycosides (digitoxin, digoxin, lanatoside C ouabain and calactin)" BR. J. PHARMACOL., vol. 42, no. 1, 1971, pages 143-152, XP002078318 see page 145	1-8
P , X	F. KIUCHI ET AL.: "Cytotoxic priciples of a Bangladesh crude drug, akond mul (roots of Calotropis gigantea L.)" CHEM. PHARM. BULL., vol. 46, no. 3, 1998, pages 528-530, XP002078319 see the whole document	1-6
	WO 92 09295 A (MRAK, M.,) 11 June 1992	
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Inte onal Application No
PCT/08/8/01522

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.(Continua	ation) DOCUMENTS CON ED TO BE RELEVANT		
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	A.E.MUTLIB ET AL.: "In vivo and in vitro metabolism of gomphoside, a cardiotonic steroid with doubly-linked sugar."  J. STEROID BIOCHEM., vol. 28, no. 1, 1987, pages 65-76, XP002078320		·
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